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# STUDIES ON THE MODE OF ACTION OF IRRADIATED ERGOSTEROL

## I ITS EFFECT ON THE CALCIUM, PHOSPHORUS AND NITROGEN METABOLISM OF NORMAL INDIVIDUALS<sup>1 2</sup>

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### INTRODUCTION

The literature dealing with the development of irradiated ergosterol has been adequately reviewed in recent articles (1-13) Steenbock (14, 15) and Hess (16) in 1924 observed that certain foods, notably oils, milk, and cereals, can be made to possess antirachitic properties by exposure to ultraviolet light It soon became evident that this acquired antirachitic property was lodged in the sterol portion of these foods, and at first it was thought that this sterol was cholesterol (17, 18, 19) By 1926 the investigations of Rosenheim and Webster (20) and of Hess and Windaus (21) had demonstrated clearly that the antirachitic properties were taken on not by cholesterol itself, but by an accompanying sterol, ergosterol This sterol had been isolated years before by Tanret (22), who differentiated it from cholesterol and extracted it from ergot, yeast, mushrooms, and other fungi

In the last five years irradiated ergosterol has been given extensive laboratory and clinical trial Neglecting the data which have been acquired as to its chemistry, physical properties, preparation and activation, one may outline the present state of knowledge concerning it as follows

(1) Irradiated ergosterol is definitely prophylactic and curative in rickets and rachitic tetany (1, 3, 8, 23) Coincident with clinical improvement, there occurs a rise in the values (if low) of serum calcium and phosphorus, and a normal deposition of lime salts in the bones The efficacy of irradiated ergosterol in osteomalacia is practically as well established, but improvement is reported to take place more slowly than in rickets (24, 25) Its reported effectiveness in hastening the union of fractured bones (26, 27), in preventing dental caries (28), in increasing bodily resist-

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ance against infections (2, 29), in preventing rickets in infants by administration to pregnant and nursing mothers (30), and in other situations of less definite indication, remains to be settled by wider use and further study

(2) Irradiated ergosterol is a very potent agent, the often quoted statement that 1 mgm has the antirachitic potency of about 200,000 times that amount of standard cod liver oil is sufficiently accurate to serve as emphasis of this strength

(3) Its administration in excessively large doses to animals (31-35) has resulted in anorexia, loss of weight and strength, often diarrhea, emaciation, and finally death. At necropsy calcium deposits have been found in various tissues, particularly the stomach wall, heart muscle, blood vessels, kidneys, bladder, ureters, and lungs (35, 36, 37). Some workers have ascribed the toxic effects of irradiated ergosterol to contained impurities (38, 39, 40).

Because no adequate explanation concerning the mode of action of irradiated ergosterol could be found in the literature previously mentioned, studies were undertaken to determine the effect of irradiated ergosterol on the calcium and phosphorus metabolism of normal individuals and individuals with various disorders of calcium metabolism. The data from these experiments and some observations on animals are here presented because they are of value in explaining the *modus operandi* of this vitamin D substance. A preliminary report of these studies has been published (58).

We are indebted to the Winthrop Chemical Company of New York for supplying us with large quantities of "Vigantol" (Irradiated Ergosterol-Winthrop) for use in these experiments. The late F. C. Waldecker of the Winthrop Chemical Company informed us that this was the same specially prepared product furnished to Shohl et al. (37) who found that "0.0001 to 0.00025 mgm is sufficient, when fed to rats on the Steenbock diet, to protect against rickets."

#### METHODS OF STUDY

Patients were studied for varying lengths of time in the Metabolism Ward. Each patient was kept on a constant fluid intake and on a diet which was accurately weighed, adequate in caloric and vitamin content and the ash of which was neutral in reaction. The mineral content of each diet was carefully estimated and kept constant throughout each experiment. The urine and stools were carefully collected in three-day periods and prepared for analysis. The collecting of excreta was not begun until the patient had been on the special diet for at least ten days. Thus the control periods represented the true excretions for the diet employed. The routine of the ward and the laboratory has been outlined in a previous paper (41). Calcium was determined by the method

of Fiske (42), phosphorus, by the method of Fiske and Subbarow (43), carbon dioxide content of blood, by that of Van Slyke (44), cholesterol, by that of Bloor, Pelkan and Allen (45), and nitrogen, by the Kjeldahl method (46)

# EXPERIMENTS

## I Normal individuals receiving small doses of irradiated ergosterol

### Experiment I

Mr R L, an apparently normal 19 year old Italian school boy, who volunteered for this investigation, was studied for 51 days while receiving a low calcium diet. During the last 33 days of this period he was given 5 mgm of irradiated ergosterol per day. In Charts 1A, 1B and Table I are presented the data.

The fecal calcium averaged 0.25 gram during the medication period compared to an average of 0.20 gram during the control periods (see Table I). The average urinary calcium values for the same periods of

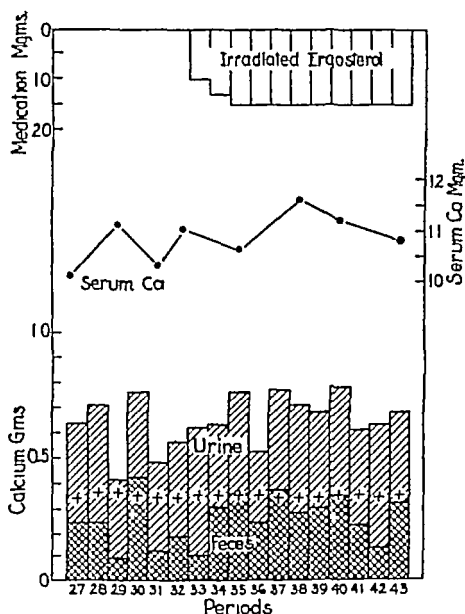


CHART 1A GRAPHIC REPRESENTATION OF CALCIUM METABOLISM IN EXPERIMENT I

In this chart as well as in all subsequent charts the intake is designated by +, the urinary excretion by single hatching and the fecal excretion by double cross hatching

TABLE I  
*The effect of irradiated ergosterol on the calcium, phosphorus and nitrogen metabolism of normal individuals*  
*Average values in grams per three day period*

Small doses of irradiated ergosterol																
Experiment	Periods	Average weight kgm	Phosphorus			Calcium			Nitrogen			Irradiated ergosterol per period	Remarks			
			In-take	Output		In-take	Output		In-take	Output						
				Urine	Feces		grams	Bal- ance		grams	grams			grams	Bal- ance	grams
I Mr R L	27 to 32	77.1	grams	grams	grams	grams	grams	grams	grams	grams	grams	mgm	Low calcium diet Average dietary formula per three day period = $C_{50} P_{110} F_{115}$ High calcium diet Average dietary formula per three-day period = $C_{125} P_{115} F_{115}$			
	33 to 43	73.8	2.40	2.23	0.38	-0.21	0.34	0.38	0.20	-0.24	35.0	33.7		3.5	- 3.0	0
			2.40	1.72	0.47	+0.21	0.34	0.41	0.25	-0.32	35.0	33.7		3.5	- 2.2	15
II Mrs M A R	15 to 18	70.2	5.14	3.02	1.25	+0.87	4.28	0.75	2.67	+0.86	47.7	31.1	4.8	+11.7	0	
	19 to 23	70.1	5.16	3.13	1.07	+0.96	4.28	0.77	2.83	+0.68	47.4	35.6	4.7	+ 7.1	30	
	24 to 27	70.3	5.16	3.08	1.16	+0.92	4.28	0.61	3.01	+0.66	44.8	36.9	4.5	+ 3.4	60	
Large doses of irradiated ergosterol																
III Mrs M A R.	1 to 6	73.6	2.10	1.38	0.49	+0.23	0.28	0.25	0.28	-0.25	29.8	23.5	3.0	+ 3.3	0	
	7 to 14	72.6	2.09	1.57	0.42	+0.10	0.27	0.39	0.17	-0.29	29.0	21.5	2.9	+ 4.6	90	
	12 to 14	72.3	2.07	1.51	0.31	+0.25	0.27	0.46	0.11	-0.30	29.0	23.1	2.9	+ 3.0	90	
IV Mr R L.	4 to 5	75.1	5.78	3.68	0.99	+1.11	4.46	0.88	1.91	+1.67	58.6	46.6	5.9	+ 6.1	0	
	6 to 14	75.7	5.78	3.67	0.93	+1.18	4.46	1.19	1.54	+1.73	58.6	42.5	5.9	+10.2	90	
	15 to 23	76.5	5.78	3.64	0.84	+1.30	4.46	1.19	1.61	+1.66	58.6	46.3	5.9	+ 6.4	30	
	24 to 26	76.9	5.78	3.60	1.25	+0.93	4.46	1.00	2.29	+1.17	58.6	53.0	5.9	- 0.3	0	

study were 0.41 gram and 0.38 gram respectively. One is hardly justified in calling such slight changes an effect of the administration of irradiated ergosterol. The serum calcium rose slightly.

The effect on the fecal phosphorus was little greater than that on the fecal calcium. The average for the control periods was 0.38 gram as compared to 0.47 gram during the medication period. The effect on the urinary phosphorus, however, was more marked, for it fell from an average of 2.23 grams in the control periods to 1.72 gram in the ergosterol periods. These changes resulted in a shift from a negative phosphorus balance of 0.21 gram to a positive balance of 0.21 gram. There occurred no variations in the serum phosphorus which could be attributed to irradiated ergosterol.

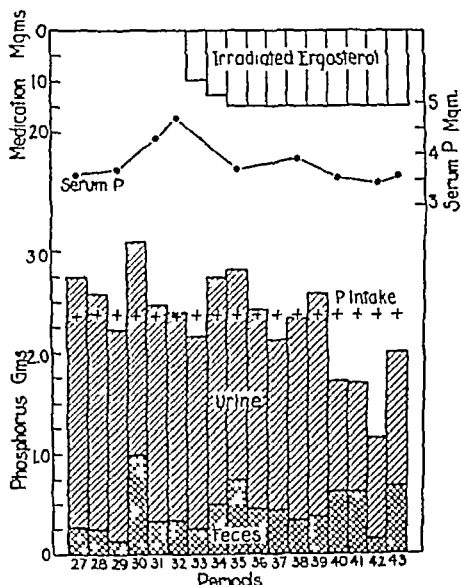


CHART 1B PHOSPHORUS METABOLISM IN EXPERIMENT I

### Experiment II

Mrs M A R, an apparently normal 27 year old widow, who volunteered for this purpose, was studied for 39 days, while receiving a *high* calcium diet. During the last 27 days of this period she was given *small* doses of irradiated ergosterol. During the first 15 days she received 10 mgm a day, for the remaining 12 days, 20 mgm. The data from this case are shown in Charts 2A, 2B and Table I.

One notes that doses of 10 and 20 mgm of irradiated ergosterol produced very little more effect on the calcium metabolism than did 5 mgm in experiment I. True, the fecal calcium rose from an average of 2.67 grams during the control periods to 2.83 grams while receiving 10 mgm a day and finally reached 3.01 grams during the periods in which 20 mgm

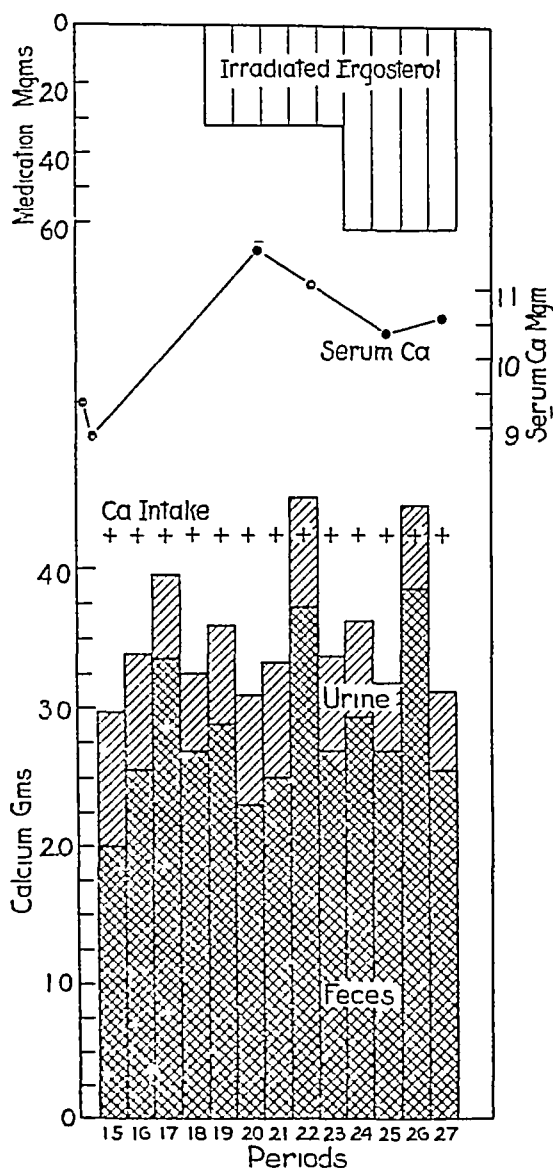


CHART 2A CALCIUM METABOLISM IN EXPERIMENT II

a day were given. Instead of rising as it had done in experiment I, the urinary calcium fell from an average of 0.75 gram to 0.61 gram per period (see Table I). The serum calcium increased, reaching its high point 5 days after the institution of irradiated ergosterol therapy. It remained above the control values during the remainder of the study period.

The changes in the phosphorus metabolism were less marked than the calcium metabolism changes. The fecal phosphorus fell from an average of 1.25 gram in the control periods to 1.16 gram in the last three medication periods. The urinary phosphorus remained unchanged, the control value was 3.02 grams compared to an average value of 3.08 grams for the last three medication periods. These changes are very slight, yet the exact opposite of those noted in the phosphorus metabolism of experiment I. The serum phosphorus was unaffected.

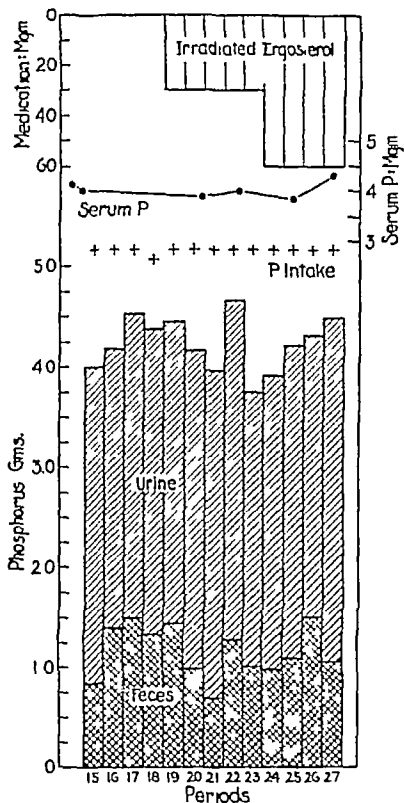


CHART 2B PHOSPHORUS METABOLISM IN EXPERIMENT II

## II Normal individuals receiving large doses of irradiated ergosterol Experiment III

Mrs M. A. R. was also studied while receiving a low calcium diet and 30 mgm of irradiated ergosterol per day. An interval of 26 days was



permitted to elapse between this period of study and the one made while the patient was on a high calcium intake. From Charts 3A, 3B and Table I, one observes that during the first period of irradiated ergosterol administration there was a marked rise in the fecal calcium and a fall in the urinary calcium. In the subsequent periods, there was a gradual fall in the fecal calcium. Coincident with this fall in fecal calcium there was a gradual rise in the urinary calcium of approximately the same magnitude. The average for the fecal calcium during the control period was 0.28 gram compared to an average of 0.11 gram during the last three periods of therapy. The urinary calcium averages for these same periods of study were 0.25 gram and 0.46 gram. There resulted a very slight increase in the already existing negative calcium balance (see Table I). The slight rise in serum calcium seen in Chart 3A probably falls within the limits of physiological variation, although it may conceivably represent an effect of irradiated ergosterol.

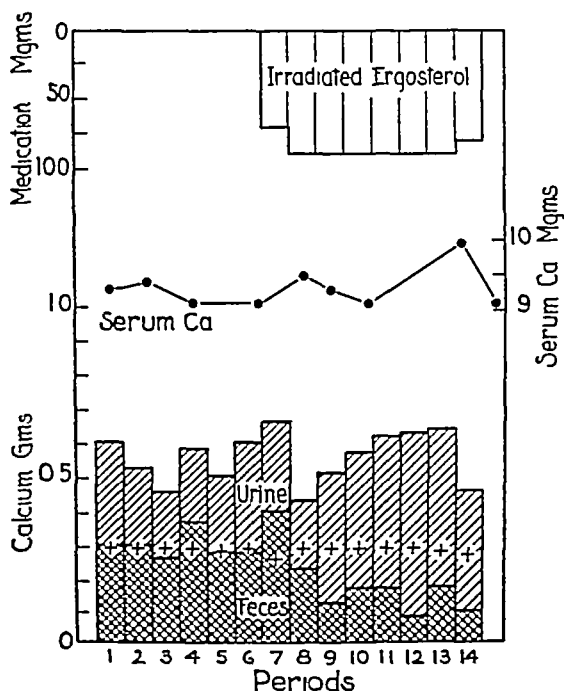


CHART 3A CALCIUM METABOLISM IN EXPERIMENT III

The effect on the phosphorus metabolism was the same as that on the calcium metabolism, although the changes were less marked. At first there was a rise in the fecal phosphorus and a fall in the urinary phosphorus. Following this there resulted a gradual fall in the fecal phosphorus. The average fecal phosphorus excretion for the control periods was 0.49 gram, falling to an average of 0.31 gram during the last three periods of therapy. The effect on the urinary phosphorus was the oppo-

site, it rose from 1.38 gram per three day period before irradiated ergosterol was administered to 1.51 gram during the last three periods of ergosterol medication. The average phosphorus balance was  $+0.23$  gram during the control period (1 to 6) compared to  $+0.10$  gram in the medication period (7 to 14), yet the average for the last three periods (12 to 14) was  $+0.25$  gram. The changes in the serum phosphorus were not constant enough to be ascribed to the effect of ergosterol.

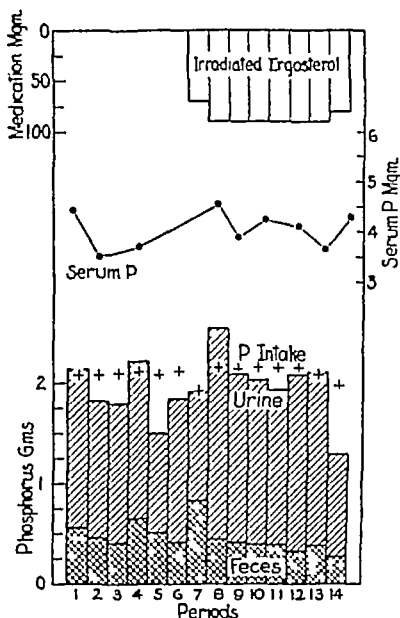


CHART 3B PHOSPHORUS METABOLISM IN EXPERIMENT III

#### Experiment IV

In Charts 4A, 4B and Table I are presented the calcium, phosphorus and nitrogen metabolism data from Mr. R. L. for a period of 78 days in which he was given a high calcium diet. During periods 6 to 14 the dose of irradiated ergosterol was 30 mgm a day. During periods 15 to 23 it was reduced to 10 mgm a day.

The calcium data confirm the observations made in experiment III (see Table I). During the 9 periods (27 days) in which 30 mgm a day were given, the fecal calcium per three-day period was 1.54 gram in contrast to the value of 1.91 gram for the control period. During this time the urinary calcium rose from a control value of 0.88 gram per three-day

period to 1.19 gram. The positive calcium balance increased from +1.67 gram to +1.73 gram. With a reduction of the irradiated ergosterol dosage to 10 mgm a day, the fecal calcium rose very slightly to 1.61 gram, while the urinary calcium remained unchanged, resulting in a

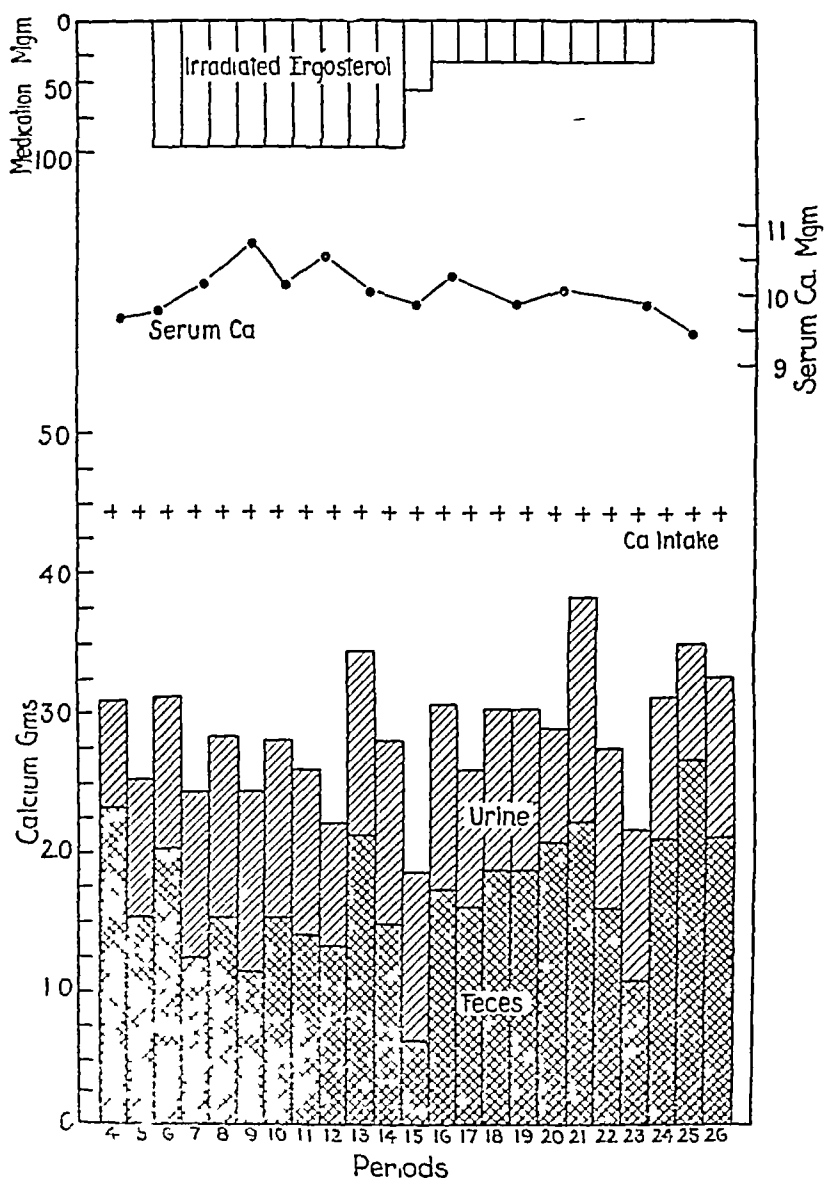


CHART 4A CALCIUM METABOLISM IN EXPERIMENT IV

slight lowering of the total calcium balance to +1.66 gram. Following the cessation of irradiated ergosterol therapy (periods 24 to 26), there was an abrupt rise in the fecal calcium to 2.29 grams, a level even higher than that observed in the control periods. The urinary calcium fell to 1.00 gram during these same two periods. The serum calcium showed changes

which are slight, but probably significant, as they parallel closely the changes in the calcium balance. The serum calcium reached a high point of 10.8 mgm per 100 cc during the period of large ergosterol dosage. On reduction to 10 mgm a day, the serum calcium fluctuated

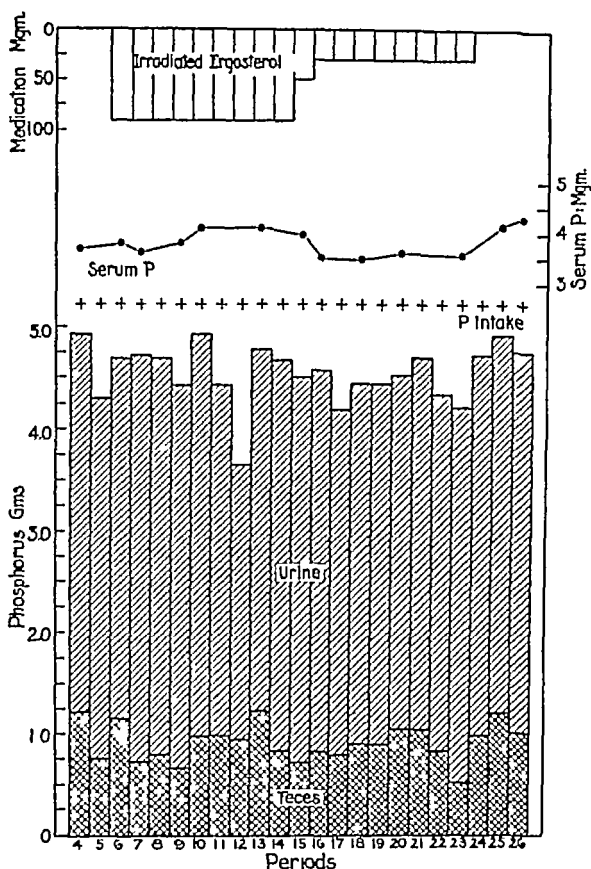


CHART 4B PHOSPHORUS METABOLISM IN EXPERIMENT IV

between 9.9 and 10.3 mgm per 100 cc. On discontinuance of the drug, it fell to 9.5 mgm per 100 cc., the value obtained during the control period.

From Chart 4B and Table I one notes that the effect on the phosphorus metabolism was not so marked. With the administration of 30

mgm of irradiated ergosterol per day, the fecal phosphorus fell to 0.93 gram as contrasted with a value of 0.99 gram during the control period. This fall continued during the subsequent periods, 15 to 23, to 0.84 gram despite the fact that the dose of irradiated ergosterol was reduced. When, however, the drug was discontinued, an abrupt and striking rise occurred. The average fecal phosphorus during these periods, 24 to 26, was 1.25 gram. The urinary phosphorus remained fairly constant during the control and medication periods, with perhaps a slight fall on discontinuance of the drug. These changes were associated with an increase in the positive phosphorus balance. The changes in the serum phosphorus were very slight, although the variations seemed to parallel those of the serum calcium.

During period 12 this patient had four or five stools a day for two days instead of his usual one or two. At the time it was thought that this might possibly be a symptom of overdosage of irradiated ergosterol. Although the same dose was continued for two more periods the bowel disturbance ceased.

### *III The effect of irradiated ergosterol administration on the absorption of large doses of calcium lactate from the gastro-intestinal tract*

In order to obtain further information concerning the effect of irradiated ergosterol administration on the absorption of calcium from the gastro-intestinal tract of normal individuals, the following experiments were performed on two normal individuals, Miss D. C. and Mr. A. M. After fasting twelve hours, each subject was given 10 grams of calcium lactate in 250 cc. of water, a control serum calcium having been obtained prior to the ingestion of the calcium lactate. Serum calcium determinations were made every hour for the first three hours and at the end of the sixth, ninth, twelfth and twenty-fourth hours. From Table II it will be noted that in each individual the serum calcium rose to a maximum height at the end of the third hour. In the case of Miss D. C. this elevation was 2.2 mgm. and in Mr. A. M., 2.5 mgm. above the fasting serum calcium value.

Irradiated ergosterol was then administered to each subject in doses of 30 mgm. a day for twelve days. On the twelfth day the same experiment was repeated. Again the serum calcium reached a maximum at the end of the third hour. However, in each subject this rise was approximately 1 mgm. higher than it had been in the control experiments.

These experiments would seem to serve as evidence that the administration of irradiated ergosterol increases the absorption of calcium from the gastro-intestinal tract. That the extra calcium absorbed is not necessarily retained is strongly suggested by the experiments performed on Mr. A. M. During each experiment the urinary calcium excretion was determined for the first twelve hours following the ingestion of the cal-

TABLE II

*Showing the effect of the ingestion of 10 grams of calcium lactate on the serum calcium before and during the administration of irradiated ergosterol*

Time	Miss D. C.		Mr. A. M.	
	Serum calcium		Serum calcium	
	Before therapy	During * therapy	Before therapy	During * therapy
Fasting	110	116	98	106
10 grams calcium lactate administered				
1 hour after	118	136	111	123
2 hours after	122	142	120	130
3 hours after	132	146	123	140
6 hours after	113	137	112	138
9 hours after	104	120	112	122
12 hours after	106	126	107	115
24 hours after	104	104	96	109

\* Each subject had received 30 mgm of irradiated ergosterol per day for twelve days. The second experiment was performed on the twelfth day of such therapy.

cium lactate. In the control experiment the amount of calcium excreted in the urine was 110 mgm, in the experiment performed during the administration of irradiated ergosterol, 195 mgm.

The experiments reported by Warkany (47b) are sufficient proof that the administration of irradiated ergosterol increases the absorption of phosphorus as well as calcium. He administered 0.5 gram of  $\text{Na}_2\text{HPO}_4$  per kilogram of body weight and noted the subsequent rise in the serum phosphorus. This same experiment was later repeated, irradiated ergosterol having been administered daily in the interim. In these latter instances, the increase in the serum phosphorus was frequently twice as great as that which he had observed in the control experiments.

#### DISCUSSION

These observations on the calcium and phosphorus metabolism of normal individuals show quite clearly the metabolic effects which result from the daily administration of irradiated ergosterol to normal individuals.

From the first two experiments it is seen that the effects of small doses of irradiated ergosterol (5 to 20 mgm) when given to normal adults on either a high or low calcium diet were very slight and not constant. The fact that the changes were very slight, inconstant and opposite in nature in these two cases probably means that the variations noted were not significant.

From experiments III and IV one notes that the effect of 30 mgm of irradiated ergosterol on normal individuals is the same whether the calcium intake is high or low. This dose exerts the same effect on both the calcium and phosphorus metabolism. *The resulting changes are a fall in the fecal calcium and phosphorus accompanied by a rise in the urinary calcium and phosphorus.* The calcium and phosphorus balances were little affected, a finding which is in accord with that of other workers (9) (48) (49). The decreased fecal calcium excretion might be interpreted as signifying either increased absorption from the gastro-intestinal tract or failure to re-excrete calcium into the bowel because of increased retention resulting from the administration of irradiated ergosterol<sup>4</sup>. The latter theory seems unjustified because of the findings in experiment III. In this instance during the control period the calcium intake and the fecal calcium were identical, 0.28 gram. The fecal calcium fell to 0.11 gram during the last three periods of irradiated ergosterol medication, although the intake remained unchanged. If this decreased fecal excretion had been due to increased retention, one would not have expected the urinary excretion to increase as much as it did, from 0.25 gram to 0.39 gram. That the increased calcium absorbed was without benefit to the body calcium stores was apparent because the negative calcium balance increased during the period of medication.

Thus it would seem that the administration of irradiated ergosterol to individuals with adequate body stores of calcium and phosphorus does not necessarily result in the retention of these elements in the body. The increased amount of calcium and phosphorus absorbed is evidently re-excreted in the urine. The fact that the urinary calcium and phosphorus rise as the fecal calcium and phosphorus fall is best interpreted as signifying that increased retention does not occur in normal individuals as the result of irradiated ergosterol administration.

The difference in the effect of small and large doses of irradiated ergosterol in normal individuals cannot be ascribed to individual variation because the same patients were used in both types of experiments.

From our experiments it would appear that the action of irradiated ergosterol is an immediate one. Beumer's (50) and Hottinger's (51) data are in accord with such a view. Gyorgy (52) stated that it took ten to fourteen days to produce a demonstrable ergosterol effect. In our experiments, the first effect noted (during the first period of its administra-

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<sup>4</sup> There is considerable evidence in the literature which proves quite conclusively that calcium which is absorbed from one portion of the bowel may be re-excreted into another portion of the gastro-intestinal tract (46, 47a, 48). Proof of this has been previously published by one of us (W. B.) (57). In a group of normal subjects (46 three-day periods in all) studied on an inadequate intake (0.33 gram per three-day period), the average fecal calcium excretion per three-day period was found to be 0.60 gram. In other words, the fecal calcium excretion was 0.27 gram in excess of the intake.

tion) was a marked rise in the fecal calcium and a fall in the urinary calcium. The fecal phosphorus and urinary phosphorus were similarly affected. Following this immediate effect there then occurred a gradual fall in the fecal calcium and phosphorus and a gradual rise in the urinary calcium and phosphorus. Hottinger (51) noted similar changes when irradiated ergosterol was administered and referred to it as the diphasic ergosterol effect. Both Hottinger (51) and Kroetz (53) thought that a state of acidosis occurred during the first period of ergosterol administration, because they observed during the first twenty four hours of such therapy an increased excretion of acid and a more acid urine. As the body returns to its normal state, the second phase sets in. We are unable to offer any explanation for this diphasic ergosterol effect observed in normal individuals.

In the normal individuals receiving small doses of irradiated ergosterol, the serum calcium was very slightly raised, although this may have been due in part in experiment II to the high calcium intake following a previous period of study on a low calcium diet. The serum phosphorus was unaffected in these two cases. When doses of 30 mgm per day were administered, the changes in the serum calcium and phosphorus were slight, but probably represented an ergosterol effect. Other workers (54) (55) have demonstrated slight rises of both the serum calcium and phosphorus in normal individuals during irradiated ergosterol therapy, whereas Havard and Hoyle (56) failed to obtain such rises. The serum calcium can be elevated to higher levels by the ingestion of 10 grams of calcium lactate during the administration of irradiated ergosterol than under normal conditions. In any condition where calcium medication is required, the daily administration of irradiated ergosterol in conjunction with large doses of a calcium salt might be a better therapeutic procedure than the administration of a calcium salt alone.

No significant changes occurred in the nitrogen metabolism in any of the four experiments (see Chart 1). The plasma cholesterol was determined repeatedly in all these experiments, but because in no instance was there any noteworthy change as a result of irradiated ergosterol administration, these cholesterol values are not included in the tables. No untoward symptoms were observed in any of the four individuals.

#### SUMMARY

1 The administration of irradiated ergosterol in *small doses* to normal individuals produced no constant changes in either the calcium or phosphorus metabolism.

2 The administration of irradiated ergosterol in doses of 30 mgm per day to normal individuals resulted in an immediate increase of the fecal calcium and phosphorus excretion. The urinary calcium and urinary phosphorus were decreased. Following this there occurred a de-



creased fecal calcium and phosphorus excretion and an increase in the urinary calcium and phosphorus excretion. The calcium and phosphorus balances were only slightly affected. Following the cessation of irradiated ergosterol administration, the fecal calcium and phosphorus promptly rose and the urinary calcium and phosphorus fell slightly.

3 The serum calcium and phosphorus of normal individuals was only slightly affected by irradiated ergosterol therapy.

4 The nitrogen excretion was unaffected by irradiated ergosterol administration.

5 No constant changes in the blood plasma cholesterol were noted during the administration of irradiated ergosterol.

6 No untoward symptoms resulted from the administration of as much as 30 mgm of irradiated ergosterol per day.

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# STUDIES ON THE MODE OF ACTION OF IRRADIATED ERGOSTEROL

## II ITS EFFECT ON THE CALCIUM AND PHOSPHORUS METABOLISM OF INDIVIDUALS WITH CALCIUM DEFICIENCY DISEASES<sup>1 2</sup>

BY WALTER BAUER AND ALEXANDER MARBLE<sup>3</sup>

(From the Medical Clinic of the Massachusetts General Hospital Boston)

(Received for publication June 6, 1931)

### INTRODUCTION

In the first paper of this series (1), we reported that the administration of adequate doses of irradiated ergosterol to normal individuals resulted in definite alterations in the fecal and urinary excretion of calcium and phosphorus. These changes consisted in a gradual fall in the fecal excretion and a gradual rise in the urinary excretion of both elements with little effect on the total balances of either. These findings are best interpreted as signifying increased absorption from the gastro intestinal tract without an accompanying increased body retention.

The fact that irradiated ergosterol acts as a curative agent in calcium deficiency diseases such as rickets and osteomalacia indicates that an increased retention of calcium and phosphorus must take place when it is administered to individuals with such diseases. In order to determine what changes occur in the calcium and phosphorus metabolism of individuals with calcium deficiency diseases when irradiated ergosterol is administered, two such patients were studied in an effort to ascertain whether the action of irradiated ergosterol was in any way different from that which we had observed in normal individuals.

### METHODS OF STUDY

These two patients were studied under the same conditions as those previously reported (1). The diets employed were the same except for calcium content. The collection and preparation of the excreta and the methods of analysis used were identical with those described in the first paper of this series (1).

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<sup>1</sup> This is publication No. 5 of the Robert W. Lovett Memorial for the study of crippling disease, Harvard Medical School, Boston, Massachusetts.

<sup>2</sup> Part of the expense of this investigation was paid by the William W. Wellington Memorial Research Fund.

<sup>3</sup> Medical Resident, Massachusetts General Hospital.



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## EXPERIMENTS

*I The effects of gradually increasing doses of irradiated ergosterol on a patient with osteoporosis**Experiment I*

Mrs M B, a 54 year old American widow, was admitted to the hospital on April 9, 1929, because of disabling pain in the back of two months' duration. X-ray examination showed a compression fracture of the vertebrae and *generalized rarefaction* of all her bones. Interestingly, her diet for the preceding six years had been almost an exact duplicate of the low calcium diet used in our previous calcium studies (2) (approximately 0.1 gram of calcium per day). Its vitamin content was very low. Other laboratory and clinical observations revealed no noteworthy abnormalities. A gastric analysis showed a normal amount of free hydrochloric acid. The only treatment employed prior to her transfer to the Metabolism Ward was that of hyperextension on a suitable frame. She was kept on a low calcium diet for six days, then on a high calcium diet for eighteen days, and finally on a high calcium diet plus irradiated ergosterol for thirty-six days. Her average weight was about 59 kgm. The metabolism data are presented in Charts 1A, 1B and Table I.

One notes that the calcium excretion on a low calcium diet, in periods 1 and 2, was very similar to that observed in normal individuals (2). The presence of normal serum calcium and phosphorus values and the finding of a normal excretion of calcium and phosphorus on a low calcium diet proved that the osteoporosis was not due to increased parathyroid activity (3-11).

During periods 3 to 8 on a high calcium diet (see Table I), the average urinary and fecal calcium values were 0.67 gram and 2.56 grams, respectively, with a positive balance of 0.70 gram. Following the administration of 5 mgm of irradiated ergosterol a day during periods 9 to 17, inclusive, the urinary calcium rose to 0.92 gram and the fecal calcium fell to 2.49 grams, thus leaving the calcium balance +0.50 gram. (Unfortunately the fecal extracts during periods 16 and 17 were lost.) During period 18, 8 mgm of ergosterol per day were given. The urinary excretion remained the same, but the fecal excretion fell to 1.48 gram and the balance rose to +1.52 gram. (These values are not so reliable as those with which they are compared, since they represent only one three-day period.) In the last two periods, 19 and 20, during which 20 mgm of irradiated ergosterol per day were given, the urinary calcium rose to +1.08 gram, the fecal calcium to 1.69 gram and the total balance was +1.15 gram. Hence irradiated ergosterol administration caused a consistent decrease in the fecal calcium and an increase of the urinary calcium to a smaller degree, with a resulting increase of the positive calcium balance.

This same effect is equally well shown in the case of phosphorus (see Table I) During the control periods the urinary phosphorus was 2.41 grams, during the subsequent periods in which irradiated ergosterol was given, it was 2.64 grams in periods 9 to 17, 2.54 grams in period 18, and 2.49 grams in periods 19 and 20. The fecal phosphorus was 1.71 gram

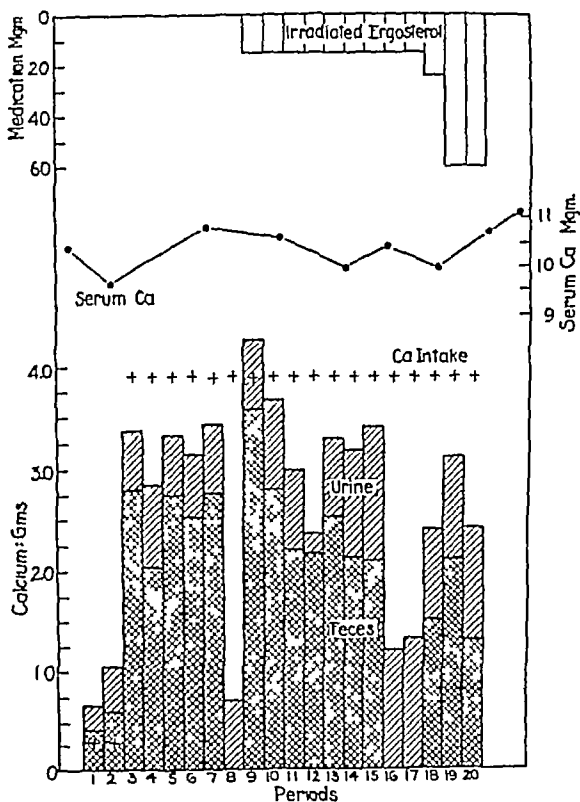


CHART 1A CALCIUM METABOLISM IN EXPERIMENT I

during the control period, this fell steadily to 1.44 gram in periods 9 to 17, 1.29 gram in period 18 and 1.12 gram in periods 19 and 20. That most of this phosphorus was retained in the body is evidenced by the fact that the increases in the urinary values were much smaller than the decreases in the fecal values. This is further borne out by the fact that the total phosphorus balances gradually increased from +0.50 gram in the control

periods to + 0.54 gram in periods 9 to 17, + 0.79 gram in period 18 and + 1.01 gram in periods 19 and 20

During the time of observation in the hospital, the serum calcium was always quite normal. One month after discharge a value of 11.1 mgm per 100 cc was obtained, which may possibly have been an ergosterol

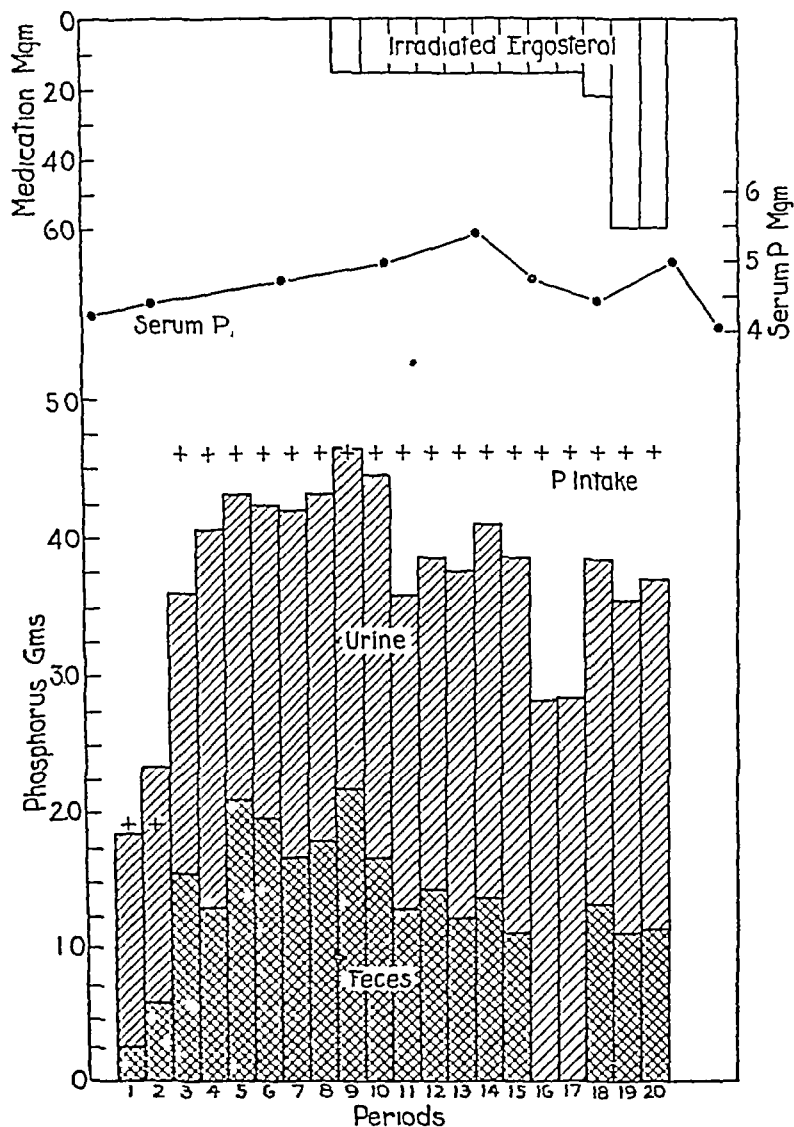


CHART 1B PHOSPHORUS METABOLISM IN EXPERIMENT I

effect, since the patient remained on a high calcium diet and continued to take irradiated ergosterol. In the serum phosphorus there occurred changes which have not been seen in other patients. Under the influence of irradiated ergosterol, the control values of 4.42 and 4.79 mgm per 100 cc rose to a high point of 5.44 mgm per 100 cc two weeks after the drug was started.

TABLE I  
*The effect of irradiated ergosterol on the calcium, phosphorus and nitrogen metabolism of individuals with calcium deficiency diseases*  
 Average values in grams per three-day period

Experiment	Periods	Average weight	Phosphorus				Calcium				Nitrogen				Irradiated steroid per period	N/C per period	Remarks
			In take	Output		Bal. ance	In take	Output		Bal. ance	In take	Output		Bal. ance			
				grams	Urine			Feces	grams			Urine	Feces				
I Mrs M B	3 to 8	38.6	4.62	2.41	1.71	+0.50	3.93	0.67	2.56	+0.70	41.6	37.5	4.2	-0.1	0	Low calcium diet during first two periods. High calcium diet in the succeeding periods. Average dietary formula per three-day period = $C_{10}H_{16}P_{10}$	cc
	9 to 17	59.1	4.62	2.64	1.44	+0.54	3.91	0.92	2.49	+0.50	44.6	38.1	4.5	+2.0	15		
	18		4.62	2.54	1.29	+0.79	3.92	0.92	1.48	+1.52	44.6	37.5	4.5	+2.6	24		
	19 to 20		4.62	2.49	1.12	+1.01	3.92	1.08	1.69	+1.15	44.6	34.4	4.5	+5.7	60		
II Mrs. De la B	24	45.3	4.82	2.11	2.69	+0.02	3.39	0.02	3.74	-0.37	41.1	43.7	4.1	-3.7	0	High calcium diet. Average dietary formula per three-day period = $C_{10}H_{16}P_{10}$	cc
	25 to 28	45.1	4.80	2.74	1.68	+0.38	3.39	0.03	2.77	+0.59	44.0	36.9	4.4	+2.7	0		
	29 to 39	45.6	4.76	2.77	0.46	+1.53	3.36	0.01	0.85	+2.47	43.9	33.3	4.4	+6.2	30		
	40 to 45	46.0	4.79	2.68	0.37	+1.04	3.39	0.03	0.93	+2.43	44.2	39.1	4.4	+0.7	50		

The blood plasma cholesterol showed no striking change, although too few determinations were made to warrant conclusions

During this period of study, the nitrogen balances gradually increased, from a control value of  $-0.10$  gram in periods 3 to 8 to  $+2.0$  grams in periods 9 to 17 and  $+5.7$  grams in periods 19 and 20. During this period of time, the patient gradually gained weight.

Clinically, marked improvement took place. The combination of prolonged treatment in hyperextension with a high calcium diet and irradiated ergosterol and subsequent braces relieved her symptoms entirely. She has been free from pain for over two years. She indulges in normal activity for a woman of her age.

From this study it is evident that a high calcium diet plus irradiated ergosterol produced an effect on the calcium and phosphorus metabolism similar to that which had been noted in normal individuals. In this case, however, the decreased fecal excretion was not accompanied by a comparable rise in the urinary excretion and as a consequence there occurred a marked increase in both the calcium and phosphorus positive balances. This might be interpreted as meaning that in an abnormal subject the extra calcium and phosphorus absorbed is retained for body needs, in the normal individual, because there is no need for calcium and phosphorus storage, the calcium and phosphorus is re-excreted in the urine. It is interesting that the positive balances increased as the dose of ergosterol was increased.

The retention of phosphorus in the body was more striking than was the retention of calcium. This may perhaps be explained on the assumption that the additional phosphorus was used by the body for the building of active tissues, this is suggested by the accompanying increase in the nitrogen balance and the slight gain in body weight.

## II *The effect of irradiated ergosterol administration on a patient with chronic diarrhea and associated tetany*

### *Experiment II*

Mrs. de la B., a 27 year old, white, married secretary, had been previously studied in this clinic. A full case report will be published elsewhere. It is sufficient to state that for about four years she had suffered from almost constant diarrhea and tetany. No therapeutic measure had been of definite or lasting value.

Further study revealed that this patient had a persistently low serum calcium ( $4.5$  mgm per  $100$  cc) and a serum phosphorus of  $1.8$  to  $2.0$  mgm per  $100$  cc. Her bones showed decreased density on x-ray examination. The diarrhea was constant. A gastric analysis proved that she had a complete absence of free hydrochloric acid, uninfluenced by subcutaneous histamine injection. Analysis of her stools revealed an abnormally high

fat content (at times 30 per cent of the wet stool) Examination of the pancreatic juice showed an abnormally low lipolytic enzyme activity The features of this case which make it possible to group it as one of tetany of the infantile type are

- 1 Low serum calcium
- 2 Low serum phosphorus (in direct contradistinction to the parathyroid type of tetany, where the serum phosphorus is high)
- 3 Decreased bone density (in contradistinction to parathyroid tetany where the bone density is normal)
- 4 Absence of cataracts (so common in parathyroid tetany) The immediate cause of the tetany was thought to be calcium and phosphorus deficiency secondary to faulty absorption Absence of free hydrochloric acid (12) (13), increased fat content of the stools (14) (15) (16) and diarrhea (14) (17) are three factors known to interfere with calcium absorption from the intestinal tract

In Charts 24 and Table I are presented the data concerning the calcium metabolism During the sixty six days of study, periods 24 to 45 inclusive, she received a high calcium diet, 3.39 grams per three-day period Her fat intake remained constant throughout this period of study

During period 24 the fecal calcium excretion was 3.74 grams, or 0.37 gram in excess of that ingested, 3.39 grams The fact that she not only failed to retain any of the ingested calcium but actually excreted calcium from her body stores is further proof that the tetany was due to failure to absorb calcium The urinary excretion was abnormally low and remained so throughout the period of observation During periods 25 to 28 inclusive, she received 18 cc. of 10 per cent hydrochloric acid a day This therapy caused a slight rise in the serum calcium and sufficient reduction in the fecal calcium excretion to enable her to remain in a slightly positive calcium balance, but was without effect on the diarrhea With the institution of irradiated ergosterol therapy in period 29 in doses of 10 mgm per day, there was a precipitous fall in the fecal calcium during the first period of its administration The lowest fecal calcium value was observed in period 33 In this period it was 0.16 gram compared to 3.74 grams in the control period This represents a decrease of 95.7 per cent

Beginning with the first period of ergosterol therapy, the serum calcium gradually rose to 8.2 mgm per 100 cc. (period 33) It remained about 8 mgm per 100 cc. throughout her stay in the hospital, and not until 3½ months later did it increase to 9.0 and 9.5 mgm per 100 cc The fact that the serum calcium was slow to reach a normal value was interpreted as being due to an attempt to replenish the body calcium stores

The dilute hydrochloric acid was discontinued in period 40 for the remainder of the study period without influencing the total calcium metabolism From period 29 through period 45 the patient remained in marked positive calcium balance.

The changes which occurred in the phosphorus metabolism (see Chart 2B and Table I) were quite as striking as those in the calcium metabolism. During the one control period, the patient was just in balance ( $+0.02$  gram) although the phosphorus intake was more than adequate for a

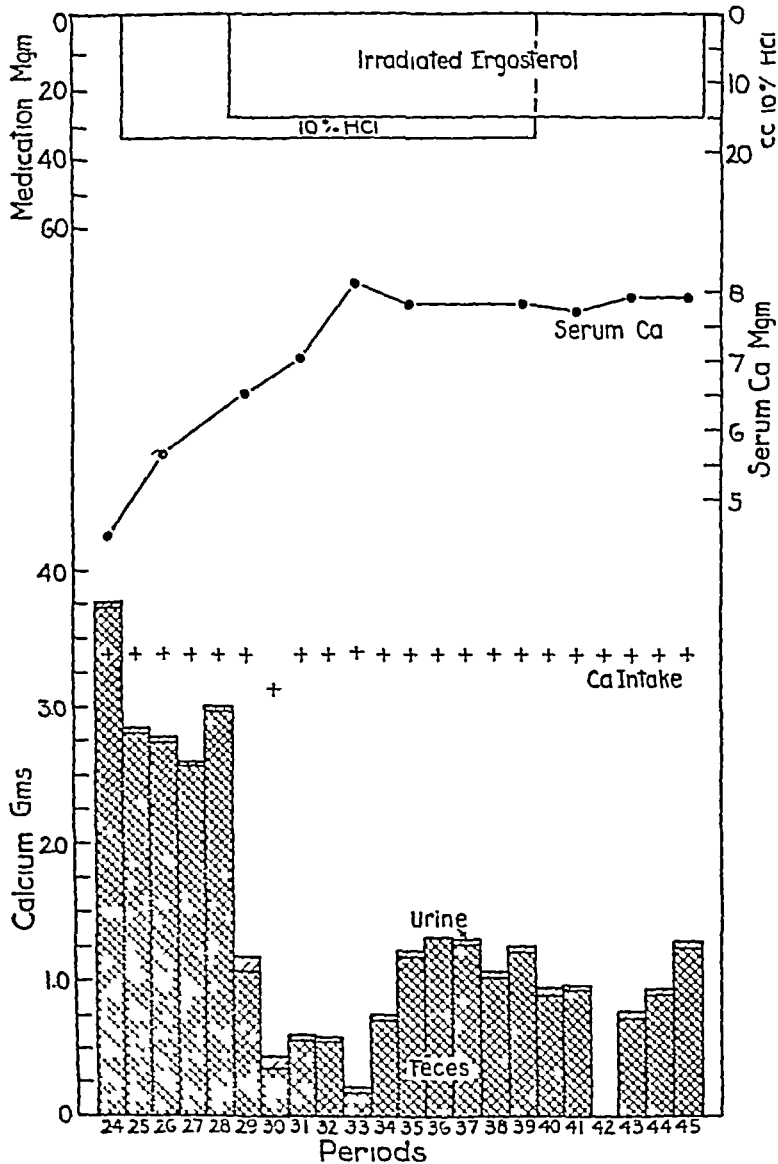


CHART 2A CALCIUM METABOLISM IN EXPERIMENT II

normal person (4.82 grams). When hydrochloric acid was given (periods 25 to 28 inclusive), the average phosphorus balance was  $+0.38$  gram. With the institution of irradiated ergosterol therapy in period 29, the fecal phosphorus fell from 1.82 gram in period 28 to 0.41 gram in period 29 and the positive phosphorus balance was greatly increased. Except

for minor variations, this beneficial effect was maintained throughout the period of study. The discontinuance of the hydrochloric acid in period 40 was without effect on the changes induced by irradiated ergosterol.

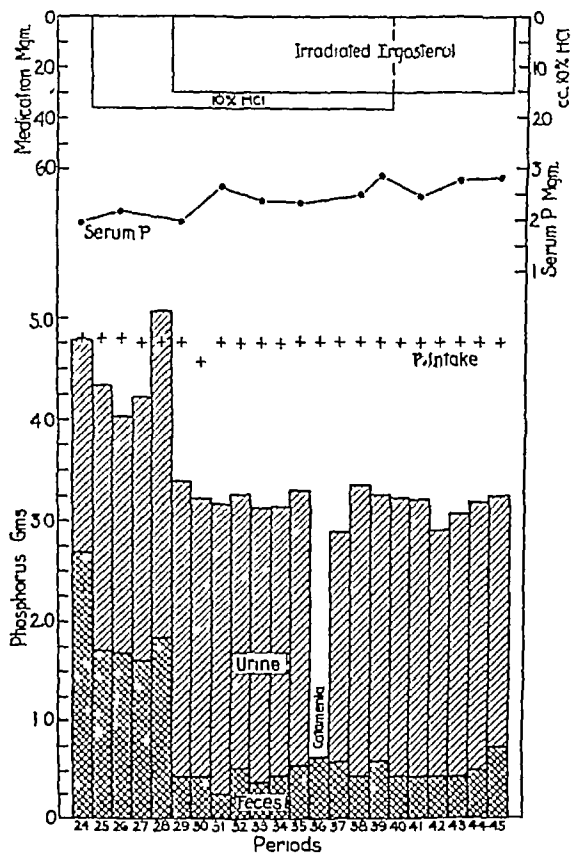


CHART 2B PHOSPHORUS METABOLISM IN EXPERIMENT II

One notes that the urinary phosphorus was high during the control period (2.11 grams) and increased during the periods of hydrochloric acid administration. The finding of a high urinary phosphorus excretion at a time when the serum phosphorus is low (2 mgm per 100 cc.) signifies that the often quoted phosphorus renal threshold of 2.8 mgm (18) (19) is not confirmed. The high urinary phosphorus excretion implies that



there was an adequate absorption from the gastro-intestinal tract of phosphorus, but, as this was not accompanied by calcium, it could not be used for bone deposition and therefore was re-excreted in the urine. The urinary phosphorus remained almost constant during the fifty-one days of ergosterol medication. Therefore, one might infer that the extra phosphorus absorbed was employed for bone deposition.

The serum phosphorus at the beginning of the experiment was 1.95 mgm per 100 cc. This value was only slightly and temporarily raised by the giving of hydrochloric acid. With the beginning of irradiated ergosterol therapy, it began to rise, but did so very slowly and only after 7½ months had it reached a normal value of 4.30 mgm per 100 cc.

In Table II, the ratios, calcium to phosphorus retained, are summarized.

TABLE II

*Mrs. de la B. Calcium and phosphorus balances and their ratio per period*

Period	Calcium balance	Phosphorus balance	Ratio Ca P	Therapy
	<i>grams</i>	<i>grams</i>		
24	-0.37	+0.02	—	Control period
25	+0.56	+0.48	1.17 1	Hydrochloric acid
26	+0.63	+0.79	0.80 1	Hydrochloric acid
27	+0.79	+0.55	1.44 1	Hydrochloric acid
28	+0.38	-0.51	—	Hydrochloric acid
29	+2.23	+1.38	1.62 1	Irradiated ergosterol
30	+2.72	+1.37	1.99 1	Irradiated ergosterol
31	+2.81	+1.61	1.75 1	Irradiated ergosterol
32	+2.82	+1.51	1.87 1	Irradiated ergosterol
33	+3.21	+1.65	1.95 1	Irradiated ergosterol
34	+2.66	+1.64	1.62 1	Irradiated ergosterol
35	+2.19	+1.48	1.48 1	Irradiated ergosterol

During the administration of irradiated ergosterol, calcium and phosphorus were retained in proportions approaching very closely those found in calcium phosphate (Ca P 1.93 1). Particularly was this true in periods 30 to 33 inclusive. In these periods the Ca P ratio varied from 1.75 1 to 1.99 1 contrasted with values of 1.17 1 to 1.44 1 in the periods before ergosterol was given. These facts plus the knowledge that the nitrogen balance and the body weight were not affected signify that little of the retained phosphorus was used in the formation of active tissue.

A slight lowering of the blood cholesterol was noted.

Thus it would appear that the tetany in this individual was due to long standing calcium and phosphorus deficiency because of faulty absorption. This difficulty of absorption was much greater in the case of calcium than of phosphorus. The interference with absorption may have been due to the excess of fat in the stools, the achlorhydria, the diarrhea, or to vitamin D deficiency.

Certain workers have attributed the calcium deficiency occurring in patients with fatty diarrhea and associated tetany to the excess of lipoids in the stool (14) (15) (16). Telfer (16) inferred that because of the excess fat excretion, calcium was bound as an insoluble soap and was therefore excreted. He contended that phosphorus was absorbed in the normal manner but could not be retained for calcium deposition without calcium and therefore was re-excreted in the urine. However, other investigators (18) (20) have shown that the formation of an insoluble soap is not the sole explanation for the inability to absorb calcium.

The restriction of fat seemed indicated in our case because of the low lipolytic ferment activity in the duodenal juice. However, restriction of the fat intake alone did not affect the absorption of either calcium or phosphorus. This is well shown in Table III, which represents periods of

TABLE III

*Mrs. de la B. Additional data on phosphorus and calcium balance and fat intake*

Period	Phosphorus					Calcium					Dried feces	Fat intake
	Output			Intake	Balance	Output			Intake	Balance		
	Urine	Feces	Total			Urine	Feces	Total				
	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams
7	1.19	1.25	2.44	3.31	+0.87	0.01	1.15	1.16	2.26	+1.10	198	328
8	1.26	0.84	2.10	3.48	+1.38	0.01	0.51	0.52	2.26	+1.74	181	208
9	0.66	1.76	2.42	3.48	+1.06	0.01	2.22	2.23	2.26	+0.03	39	165
10	1.20	0.88	2.08	3.37	+1.29	0.03	1.04	1.07	2.26	+1.19	104	187
11	1.42	2.13	3.55	3.31	-0.24	0.02	2.80	2.82	2.26	-0.56	93	198
24	2.11	2.69	4.80	4.82	+0.02	0.02	3.74	3.76	3.39	-0.37	—	123

study not presented in Charts 2A, 2B or Table I. It is obvious that the absorption of neither calcium nor phosphorus seemed to bear any relation to the fat intake. These findings, plus the fact that Linder and Harris (18) obtained striking improvement in two out of three similar cases before any dietary restrictions were employed, would allow one to question the statement that the restriction of fat is of prime importance in the treatment of this syndrome (18).

The administration of dilute hydrochloric acid while on a low fat intake caused only a very slight positive calcium and phosphorus balance and was without effect on the diarrhea.

However, the administration of small doses of irradiated ergosterol acted as a specific, produced a marked fall in the fecal calcium and phosphorus with a marked increase of the calcium and phosphorus balances. The urinary calcium remained unchanged. The failure to obtain an increase in the urinary calcium was due to the fact that the serum calcium never approached the renal threshold level for calcium (19).

The serum phosphorus rose much later than the serum calcium, requiring  $7\frac{1}{2}$  months to reach the normal value. Linder and Harris (18)

concluded that their cases were due to vitamin D deficiency and accepted Bergeim's theory (21) as to the mode of action of irradiated ergosterol. Bergeim stated that vitamin D, promoting the breakdown of organic tissue phosphorus, causes the serum phosphorus to rise, the increased absorption and deposition of calcium being secondary to this process. Such an explanation is not in agreement with the findings in this case, because the serum phosphorus did not rise above 2.8 mgm during the fifty-one days of study. Furthermore, there was no such increase in the nitrogen excretion as might be expected from the breakdown of organic tissue.

The findings in our case show that the calcium and phosphorus deficiency resulted from faulty absorption due to vitamin D deficiency. We are unable to state definitely that the vitamin D deficiency was secondary to the fatty diarrhea. It is conceivable that most of the vitamin D-containing sterols were excreted with the excess fat. Such an hypothesis was put forth by Linder and Harris (18). It was thought that the fatty diarrhea in this case was probably due to decreased external pancreatic secretion, as evidenced by the low lipolytic ferment activity in the duodenal juice.

#### COMMENT

Comparison of these data with those obtained in the study of normal individuals reveals that in all instances the administration of irradiated ergosterol caused an increased absorption of calcium and phosphorus from the gastro-intestinal tract. However, in the case of individuals with calcium and phosphorus deficiency diseases the absorption of calcium and phosphorus was greater, the accompanying rise in the urinary calcium and phosphorus excretions was less and, consequently, the retention of calcium and phosphorus was definitely increased.

The observed differences in the calcium and phosphorus metabolism of normal individuals and individuals with calcium and phosphorus deficiency diseases following the administration of irradiated ergosterol are easily explained if one accepts the division of disorders of calcium and phosphorus metabolism recently proposed by Albright, Bauer, Cockrill and Ellsworth (22). These authors assumed that normally the circulating fluid contains all the calcium phosphate it can hold at that particular time and that calcification is somewhat analogous to the precipitation of a calcium salt due to some local change in the environment which favors precipitation. They divided all disorders of calcium and phosphorus metabolism into three fundamental groups, viz

1. Conditions in which the body fluids are deficient compared with normals in respect to calcium phosphate and as a result there is a failure of deposition of calcium phosphate in the osteoid matrix of bone, as in the case of rickets or osteomalacia.

2 Conditions in which the body fluids contain more than the normal amount of calcium phosphate In such conditions calcium phosphate is deposited in tissues other than bone, osteoclastic tumors and irradiated ergosterol poisoning being examples of this group

3 Conditions in which the body fluids contain the normal amount of calcium phosphate, but the relation of calcium to phosphorus is abnormal, as in hypo and hyperparathyroidism

With these facts in mind it is easy to understand why non toxic doses of irradiated ergosterol when administered to normal individuals do not produce greater changes in the calcium or phosphorus metabolism In normal individuals the body stores of calcium and phosphorus, the bones, are adequate and the circulating fluid already contains all the calcium phosphate it can hold at that particular time Therefore, the additional calcium and phosphorus absorbed from the gastro intestinal tract is rapidly excreted in an attempt to maintain the normal relation of calcium to phosphorus in the serum

The patient with osteoporosis was similar to the normals in respect to the relation of calcium to phosphorus in the serum, but the body stores of calcium and phosphorus were greatly depleted Evidently the lack of calcium phosphate in the bones was sufficiently great to allow more deposition of calcium phosphate than would normally take place, and in consequence, the amount of calcium and phosphorus which had to be excreted in the urine in order to maintain a normal serum calcium phosphate concentration was less than that observed in normal individuals Therefore, the additional calcium and phosphorus absorbed because of the administration of irradiated ergosterol had only a slight effect on the serum calcium and phosphorus

In patients such as the one studied in experiment II, the body fluids were deficient in respect to calcium phosphate and therefore deposition of calcium phosphate in the bones did not take place In this patient the additional calcium and phosphorus absorbed following the administration of ergosterol was employed in restoring the normal relation of calcium to phosphorus in the serum, thus allowing calcium phosphate deposition in the bones to occur The existing calcium phosphate deficiency in the body fluids and bones was so great that there was no need of increased urinary calcium and phosphorus excretion in order to maintain the normal relations as in the case of normal individuals or the patient with osteoporosis

These findings make it clear why irradiated ergosterol therapy produces such beneficial results in calcium deficiency diseases such as rickets and osteomalacia These are diseases in which the calcium deficiency is due to failure to absorb sufficient calcium and phosphorus from the gastro intestinal tract

## CONCLUSIONS

1 A case of osteoporosis was greatly improved by the administration of irradiated ergosterol in conjunction with a high calcium diet

2 A case of osteomalacia with tetany secondary to faulty absorption showed immediate and lasting benefit from irradiated ergosterol. Irradiated ergosterol acts as a specific drug in this type of calcium phosphate disorder

3 No untoward symptoms were observed in either case as the result of such therapy

4 The action of irradiated ergosterol is the same in normal individuals as in individuals with calcium deficiency diseases, namely to increase absorption of calcium and phosphorus from the gastro-intestinal tract. If there exists a calcium phosphate deficiency of the serum as well as the bones, the additional calcium and phosphorus absorbed is retained in order to establish a normal relationship of calcium and phosphorus in the serum and thus allow the deposition of calcium phosphate in the bones. In calcium deficiency diseases where there is a calcium phosphate deficiency of the bones only, the consequent retention is not so marked, whereas in normal individuals little or no retention takes place because the serum and bones are both normal in respect to calcium phosphate

5 The dose of irradiated ergosterol necessary to produce such changes in the calcium and phosphorus metabolism of individuals with calcium deficiency diseases is much smaller than the amount required to produce changes in the calcium and phosphorus metabolism of normal individuals

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# STUDIES ON THE MODE OF ACTION OF IRRADIATED ERGOSTEROL

## III THE EFFECT OF IRRADIATED ERGOSTEROL ADMINISTRATION ON THE FORMATION OF BONE TRABECULAE<sup>1</sup>

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In a previous communication (1) data were presented which showed that the administration of irradiated ergosterol to individuals with calcium deficiency diseases resulted in an increased absorption and retention of calcium and phosphorus Presumably these retained salts were deposited in the bones The amount of retention noted during such therapy apparently depended upon the severity of the existing calcium phosphate deficiency If there existed a calcium phosphate deficiency of the serum as well as of the bones, the retention of calcium and phosphorus was greater than when there was a calcium phosphate deficiency of the bones only

The present experiments were undertaken in order to obtain direct proof that the calcium and phosphorus retained in calcium deficiency diseases subsequent to irradiated ergosterol therapy was deposited in the bones It was also hoped that it could be demonstrated that the deposition of calcium phosphate in such cases was more marked when irradiated ergosterol was administered in conjunction with a high calcium diet than when only a high calcium intake was employed

### *I Adult cats previously on a low calcium diet*

Calcium phosphate deficiency of the bones can be produced in cats by keeping them on a diet inadequate in calcium for a period of months (2) This calcium phosphate deficiency is characterized by a depletion of the bone trabeculae Therefore, these experiments were carried out on cats which had received a diet low in calcium for a period of months and, as a consequence, exhibited signs of calcium phosphate deficiency

Twelve cats were studied Ten had been on an inadequate calcium intake for 7½ months, two for 22 months At the end of such periods, the

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left fore leg of each cat was amputated at the shoulder joint under ether anesthesia. The humerus of each leg was prepared as previously described (2). Each humerus showed the characteristic depletion of the bone trabeculae. The humeri were saved until the cats were sacrificed so that a comparison could be made with the corresponding humerus of the opposite leg. Following the operation, the diet was changed from one inadequate in calcium to one having a high calcium content.

Six of the animals were given varying doses of irradiated ergosterol by mouth. The other six were kept as controls, receiving only the high calcium diet. The animals were grouped into pairs as nearly equal in size and weight as possible, one to serve as a control animal and one to receive irradiated ergosterol. The paired animals were simultaneously sacrificed under ether anesthesia at varying lengths of time following the institution of the high calcium diet. The humeri of the right fore legs were saved for examination. The preparation of all bones was identical. Some animals received irradiated ergosterol for 8 days and others for as long as 57 days. Accurate account was kept of the food intake. As may be seen in Table I, the average calcium intake of the cats receiving ergosterol was less than that of their control mates, in some instances only half. Despite this, the bones of the cats receiving irradiated ergosterol showed definitely more trabeculae than did those of their control mates (see Plates I and II). With the exception of cat 11, the cats receiving irradiated ergosterol showed in every instance a higher serum calcium than their control mates and three of these same cats showed a lowered serum phosphorus. If overdosage is the cause of these findings it did not persist long enough to produce the demonstrable decalcification which has been reported by other workers (3), (4), (5).

In cats 11 and 12, the results obtained were disappointing. Before the experiment these two cats, which had been on a low calcium diet for almost two years, exhibited signs of calcium phosphate deficiency of both the serum and the bones: tetany, a low serum calcium, and a marked decrease in the number of bone trabeculae. Yet the humerus of cat 11, after the high calcium diet—irradiated ergosterol regime, did not show appreciably more trabeculae than did the humerus of cat 12, after the high calcium diet period without irradiated ergosterol therapy. Although the serum calcium of the cat receiving irradiated ergosterol had increased from 8.2 mgm. to 11 mgm., it was not so high as the serum calcium of the control animal (12 mgm. per 100 cc.). The failure to obtain more striking differences as the result of irradiated ergosterol therapy can probably be explained by the short duration of the experiment, and the failure of cat 11 to eat the high calcium diet employed.

These experiments furnish definite evidence that the increased calcium and phosphorus absorbed during the administration of irradiated ergosterol results in the deposition of calcium phosphate in the bones.

TABLE I

*Record of the weight average daily dose of irradiated ergosterol length of time it was administered the food intake and the serum calcium and phosphorus on the day the cats were sacrificed*

Cat number and sex	Date	Weight	Irradiated ergosterol		Average intake (per day)		Serum			Number of control mate
			Length of the experiment	Per day	Calcium	Phosphorus	Date of termination	Calcium	Phosphorus	
		kgm	days	mgm	grams	grams		mgm per 100 cc	mgm per 100 cc.	
I ♂	June 11 July 2	3.35 3.20	36	None	6.69	7.99	July 17	10.3	6.78	VI
VI ♂	June 11 July 2	3.35 3.15	36	1.8	2.69	3.19	July 17	12.5	5.32	I
II ♂	June 11	2.20	8	3.0	1.39	1.07				None
III ♂	June 11 July 2 August 7	2.35 2.40 2.05	51	None	13.08	10.12	August 7	12.0	6.18	V
V ♂	June 11 July 2 August 7	3.05 2.50 2.75	57	1.0	5.88	4.97	August 7	17.2	4.46	III
IV ♀	May 25 June 11	3.05 3.05	8	3.0	1.07	1.85				None
VIII ♀	June 11 July 2 July 24	2.80 2.75 2.65	43	1.6	10.43	11.80	July 24	14.6	7.39	IX X
IX ♀	June 11 July 2 July 24	2.10 1.90 1.70	43	None	8.38	6.48	July 24	11.7	5.09	VIII X
X ♂	June 11 July 2 July 24	4.00 4.20 3.55	43	None	14.95	15.24	July 24	9.8	6.54	VIII IX
XI ♀	July 10 August 7	2.59 1.80	20	1.0	1.96	1.52	July 10 August 7	8.2 11.0	5.81 3.65	XII
XII ♀	July 10 August 7	2.39 1.90	20	None	5.24	4.06	August 7	12.1	6.63	XI

\* On low calcium diet for 22 months all other cats on low calcium diet for 7½ months prior to change to adequate calcium diet and (in some cases) irradiated ergosterol

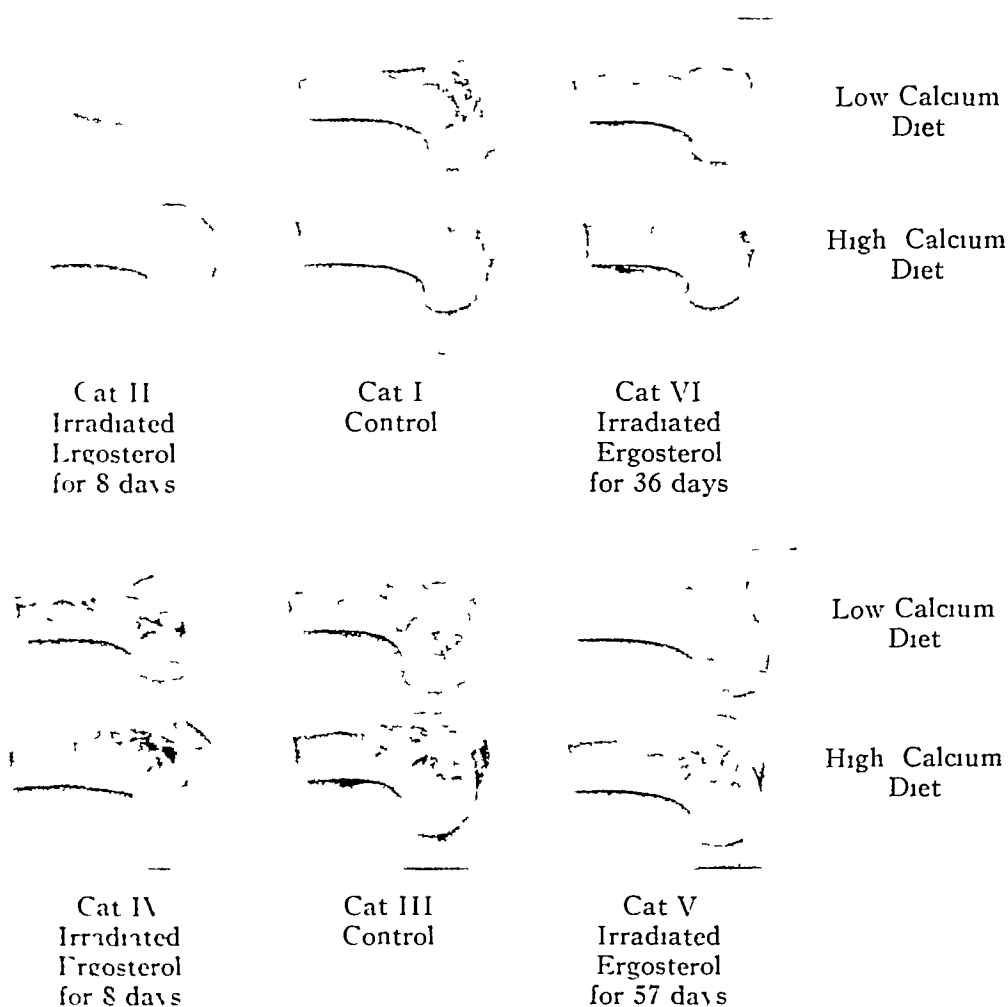


PLATE I SHOWING THE EFFECT OF IRRADIATED ERGOSTEROL ON THE FORMATION OF BONE TRABECULAE IN CATS PREVIOUSLY ON A LOW CALCIUM DIET

The fact that the number of bone trabeculae was slightly greater in the animals receiving irradiated ergosterol in addition to a high calcium diet is of much greater significance when one remembers that the average calcium intake for these animals was considerably less than the intake of the control animals. The increase in the number of bone trabeculae is not so apparent in the photographs as in the bones themselves because of the presence of high lights and shadows. This comment applies also to Plate II.

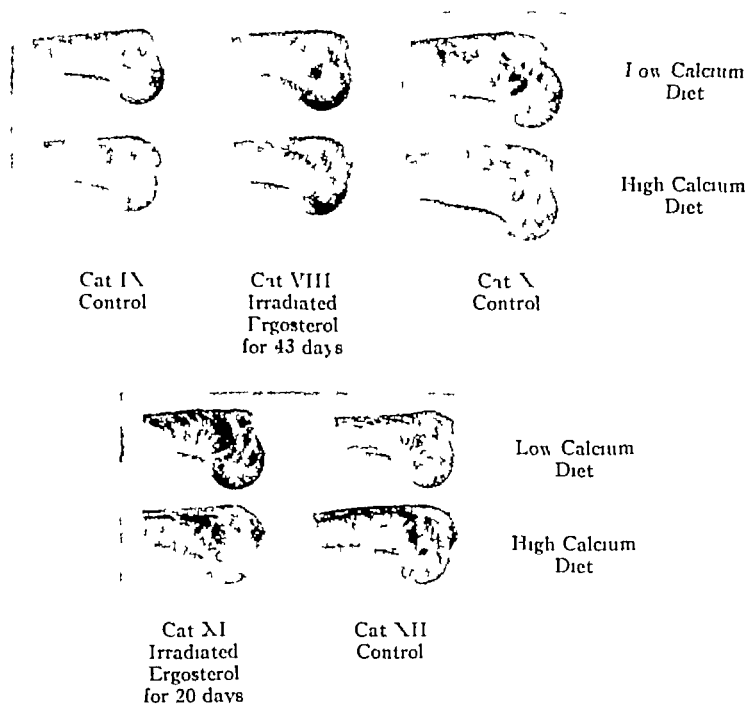


PLATE II SHOWING THE EFFECT OF IRRADIATED ERGOSTEROL ON THE FORMATION OF BONE TRABECULAE IN CATS PREVIOUSLY ON A LOW CALCIUM DIET

They also demonstrate that this deposition is greater in cases of calcium deficiency when irradiated ergosterol therapy is given in conjunction with a high calcium diet than when only a high calcium diet is employed. The results are of more significance when one realizes that the cats receiving irradiated ergosterol did not eat so much of the high calcium diet as their control mates.

## II Normal kittens

Eight kittens, 6 of them from one litter (5½ weeks old) and 2 from another litter (3 months old), were given a normal diet, adequate in calcium. Four of them were given irradiated ergosterol by mouth, in doses varying from 0.25 to 1.5 mgm a day. As set forth in Table II, kitten 13 was

TABLE II

*Record of the weight a cage daily dose of irradiated ergosterol, length of time it was administered and the serum calcium and phosphorus on the day the kittens were sacrificed*

Cat number and sex	Age at the beginning of the experiment	Date	Weight	Irradiated ergosterol		Serum			Number of control mate
				Length of the experiment	Per day	Date of termination	Calcium	Phosphorus	
	<i>months</i>		<i>kgm</i>	<i>days</i>	<i>mgm</i>		<i>mgm per 100 cc</i>	<i>mgm per 100 cc</i>	
XIII ♀	3	July 18 August 15	1.20 1.15	28	0.27	Aug 15	10.6	7.77	XIV
XIV ♂	3	July 18 August 15	1.65 1.70	28	None	Aug 15	10.6	8.63	XIII
XV ♀	1½	July 18 September 14	0.6 1.35	90	None	Oct 21	10.0	9.42	XVI
XVI ♂	1½	July 18 August 21 September 14	0.65 1.20 1.75	90	0.89	Oct 21	9.5	8.89	XV
XVII ♀	1½	July 18 September 14 September 20	0.6 1.35 1.35	63	None	Sept 20			XVIII
XVIII ♂	1½	July 18 August 21 September 14 September 20	0.6 1.0 1.30 1.35	63	0.65	Sept 20			XVII
XIX ♀	1½	July 18 August 15	0.65 1.10	28	None	Aug 15	10.1	8.28	XX
XX ♂	1½	July 18 August 12	0.65 0.95	28	0.27	Aug 15	10.1	5.87	XIX

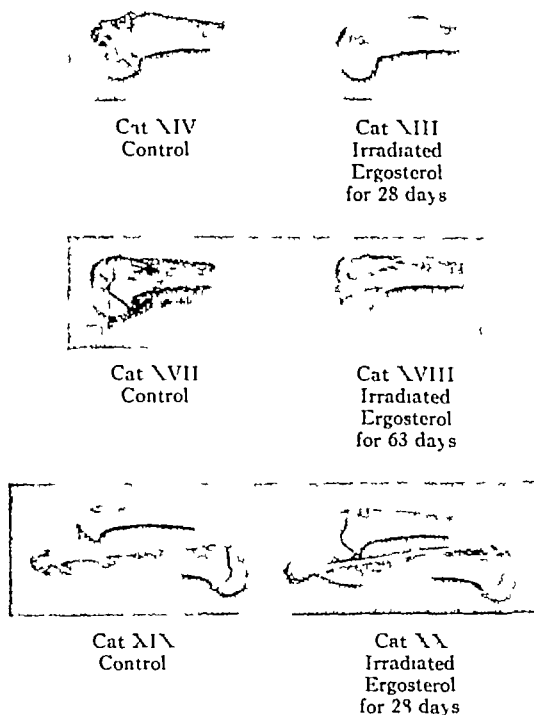


PLATE III SHOWING THE EFFECT OF IRRADIATED ERGOSTEROL ON THE FORMATION OF BONE TRABECULAE IN NORMAL KITTENS

It will be noted that the changes in these experiments were not so marked as those concerning the adult animals

paired with kitten 14, 15 with 16, 17 with 18, and 19 with 20. Kittens 13, 16, 18, and 20 were given irradiated ergosterol. They were sacrificed in pairs (except in the case of 17 and 18) as described in the previous experiment, at times varying from 28 to 63 days after the beginning of ergosterol therapy. Here, as before, increased bone trabeculation was found in the kittens receiving irradiated ergosterol (see Plate III). However, no difference was demonstrated in the level of the serum calcium, although the serum phosphorus of the animals receiving irradiated ergosterol was lower.

The findings in these experiments are in accord with those of the other experiments described in this paper, except that the changes are not so marked.

#### COMMENT

Previous experiments (1) demonstrate that calcium and phosphorus absorption and retention is increased in calcium deficiency diseases when irradiated ergosterol is administered. The present experiments show that this retained calcium and phosphorus is deposited in the bones as calcium phosphate. These experiments also demonstrate that the retention and deposition of calcium phosphate on a high calcium diet is greater in calcium deficiency diseases when irradiated ergosterol is administered. This increased retention of calcium and phosphorus is secondary to increased absorption and deposition and would not occur if a calcium phosphate deficiency of the bones did not exist. These findings are in agreement with those of Brown and Shohl (3) and Light et al. (5). These workers reported an increase in the calcium content of bone and a heavier bone ash when non-toxic doses of irradiated ergosterol were given.

The fact that the number of bone trabeculae in kittens can be increased with irradiated ergosterol therapy indicates why such treatment protects against rickets.

#### SUMMARY

1. The administration of a high calcium diet to cats with calcium phosphate deficiency of the bones results in an increase in the number of bone trabeculae.

2. This increase in the number of bone trabeculae is greater when, in addition to a high calcium diet, irradiated ergosterol is given.

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# STUDIES ON THE MODE OF ACTION OF IRRADIATED ERGOSTEROL

## IV IN HYPOPARATHYROIDISM<sup>1 2</sup>

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(Received for publication June 6 1931)

The hypothesis has been advanced that irradiated ergosterol acts only by stimulating the parathyroid glands (1-3). Yet various workers have reported beneficial results in the treatment of parathyroid deficiency with irradiated ergosterol (1), (3), (4-10). It is difficult to believe that if irradiated ergosterol acts by stimulating the parathyroid glands, its administration to completely parathyroidectomized animals should result in alleviation of the tetany and restoration of the serum calcium and phosphorus to normal values. The lack of complete calcium and phosphorus metabolism data, failure to state the dietary intake, and variations in dosage as well as differences in potency of the irradiated ergosterol preparations employed, make it difficult to draw any conclusions from the work reported in the literature as to the mode of action of irradiated ergosterol in parathyroid tetany. The results obtained in certain instances may very well have been due to overdosage of this potent vitamin D preparation (8).

In previous communications (11-13), data have been presented which were interpreted as signifying that irradiated ergosterol acted by increasing the absorption of calcium and phosphorus from the gastro-intestinal tract. If such an interpretation of the data is correct, then the beneficial results obtained in the treatment of parathyroid deficiency must bear a direct relation to the calcium intake. In an attempt to substantiate or disprove such a theory, calcium and phosphorus metabolism experiments were carried out on patients with parathyroid tetany, on varying calcium intakes, with and without irradiated ergosterol therapy. The phosphorus intake was kept constant throughout each experiment.

Three cases of hypoparathyroidism, two with idiopathic and one with postoperative parathyroid tetany, were studied. They all showed the characteristic features of this disease syndrome, namely the signs and

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<sup>1</sup> This is publication No. 7 of the Robert W. Lovett Memorial for the study of crippling disease, Harvard Medical School Boston, Massachusetts.

<sup>2</sup> Part of the expense of this investigation was paid by the William W. Wellington Memorial Research Fund.

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symptoms of tetany, low serum calcium, high serum phosphorus, and bones which appeared on x-ray examination to be of normal density. The calcium and phosphorus content of the diet given each individual was kept constant throughout each experiment. The total calcium intake was varied by administering calcium in the form of calcium chloride. One patient (Mr. B. W., experiment I) was maintained on a diet adequate in calcium, 2.08 grams per three-day period, the others received an inadequate calcium intake, 0.30 gram per three-day period. It was hoped that by this method of study it would be possible to determine whether or not the effects obtained were secondary to the calcium intake or to stimulation of remaining parathyroid tissue.

### METHODS

These three patients were studied under the same conditions as those previously described (11-12). The collection and preparation of the excreta and the methods of analyses used were identical with those employed in studies I and II (11-12).

### EXPERIMENTS

#### *Experiment I*

Mr. B. W., a 54 year old retired Jewish tailor, had suffered from idiopathic parathyroid tetany for five years. His data are presented in Charts 1A, 1B, and Table I.

During the control periods (49-51), his dietary calcium intake was 2.08 grams per period, in addition he received 36 cc. of a 45 per cent solution of calcium chloride<sup>4</sup> each period, his average calcium intake per period being 7.79 grams. The average total calcium excretion was 4.71 grams, 0.28 gram in the urine and 4.43 grams in the feces, the average positive balance being 3.08 grams. Beginning with period 52, he received 5 mgm. of irradiated ergosterol a day. During the first period of medication a rise in the fecal calcium and a fall in the urinary calcium occurred, unexplained phenomena which have been observed in previous studies (11). In the subsequent periods (53-57) the fecal calcium fell to such a degree that, although the urinary calcium rose, the positive calcium balance increased. The calcium intake was reduced during the next two periods (58-59) and the effect on the calcium metabolism is well shown in Table I. During periods 60-64, calcium chloride was discontinued, resulting in a further reduction of the calcium excretion and the positive calcium balance. When, however, the calcium intake was again raised to 7.90 grams by the administration of calcium chloride (periods 65-66), the resulting positive calcium balance greater than that in periods 52-57. For the intake and the ergosterol dosage were the same. The urinary calcium was higher, the fecal calcium lower.

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<sup>4</sup> The calcium chloride solution employed contained 162 mgm. of calcium

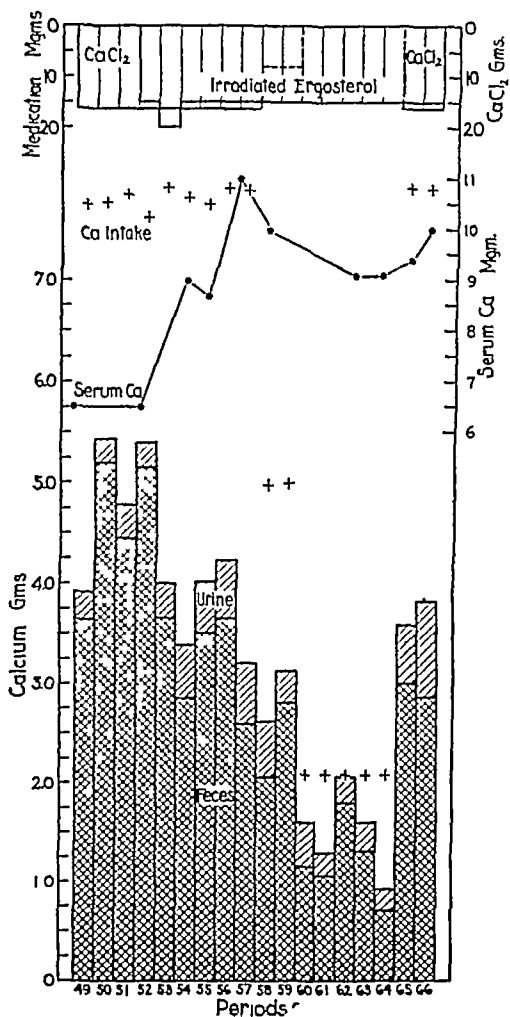


CHART 1A CALCIUM METABOLISM IN EXPERIMENT I

The changes in the phosphorus metabolism following the administration of irradiated ergosterol were a gradual increase in both the urinary and fecal values and consequently a decrease in the phosphorus balance. See Table I. These changes will be discussed in more detail below.

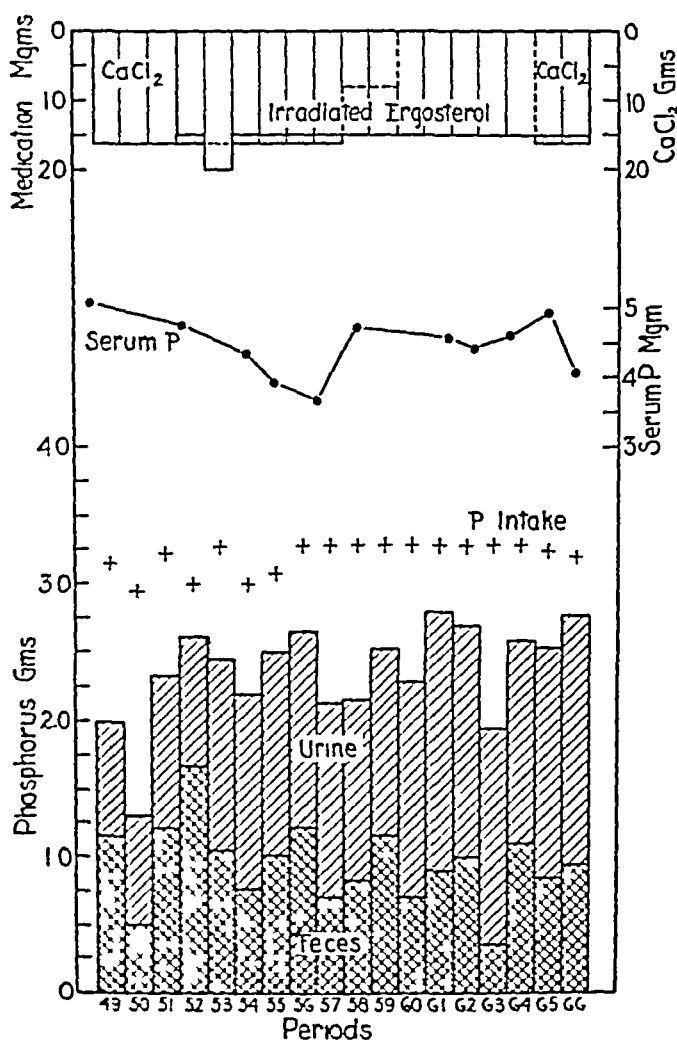


CHART 1B PHOSPHORUS METABOLISM IN EXPERIMENT I

The most interesting and significant changes noted in this experiment were those of the serum calcium and serum phosphorus. During the pre-medication periods on the high calcium intake the serum calcium was 6.5 mgm per 100 cc and the serum phosphorus was 5.08 mgm per 100 cc. These serum values remained unchanged during the first day of irradiated ergosterol administration. On the eighth day of this therapy, period 54, the serum calcium was 9.04 mgm per 100 cc and the serum phosphorus was 4.32 mgm per 100 cc. It was during this period that the urinary calcium rose, but the fecal calcium fell to such a degree that a marked

TABLE I  
*The effect of irradiated ergosterol on the calcium, phosphorus and nitrogen metabolism of individuals with parathyroid tetany*

Experiment number	Periods	Average weight kgm.	Phosphorus				Calcium				Nitrogen				Blood CO <sub>2</sub>	Treatment	
			In- take	Output		Bal- ance	In- take	Output		Bal- ance	In- take	Output		Bal- ance			
				grams	Urine			Feces	grams			Urine	Feces			grams	Urine
I. Mr B. W.	49 to 51	55.6	3.11	1.02	0.85	+1.24	7.79	0.28	4.43	+3.08	23.3	17.3	2.6	3.4	54		5
	52 to 57	55.8	3.15	1.35	1.08	+0.72	7.82	0.47	3.57	+3.78	26.0	19.2	2.7	4.1	53	54	5
	58 to 59	56.1	3.28	1.34	1.00	+0.94	4.99	0.43	2.44	+2.12	27.1	18.7	2.7	5.7	57		5
	60 to 64	56.4	3.28	1.64	0.82	+0.82	2.07	0.28	1.25	+0.54	27.1	15.6	2.7	6.8	62	64	5
	65 to 66	56.5	3.22	1.77	0.88	+0.57	7.90	0.82	2.88	+4.20	26.8	20.0	2.7	4.1	61		5
II. Master A. P.	28 to 30	22.1	1.66	0.49	0.64	+0.53	9.05	1.36	5.09	+2.60	24.3	19.2	2.4	2.7	36	24.3	3
	31 to 36	22.4	1.66	0.59	0.63	+0.44	9.05	1.35	4.17	+3.33	24.3	18.3	2.4	3.6	42	49	4
	40 to 41	22.8	1.66	0.82	0.18	+0.66	0.30	0.31	0.56	+0.37	24.3	18.9	2.4	3.0	60		3
	42 to 44	23.2	1.66	0.99	0.58	+0.09	8.83	1.55	1.92	+5.36	24.3	21.7	2.4	0.2	46		4
	1 to 5	53.2	2.09	0.49	0.41	+1.19	0.30	0.06	0.26	+0.02	28.3	13.5	2.8	12.0	None		5
III. Miss K. R.	4 to 7	53.3	2.06	0.71	0.46	+0.89	0.28	0.09	0.30	+0.11	28.0	17.9	2.8	6.3	None		5
	8 to 10	53.1	2.06	0.78	0.59	+0.69	9.03	0.34	2.61	+6.08	28.0	21.7	2.8	3.5	24.3		5*

\* No irradiated ergosterol on last two days of study

increase in the positive calcium balance occurred. When one compares the phosphorus metabolism in this period with that of the averages for the control periods, he finds that the fecal phosphorus was lower and the urinary phosphorus higher. During period 56, the fifteenth day of therapy, the serum calcium was 11.0 mgm and the serum phosphorus was 3.69 mgm. The calcium and phosphorus metabolism changes were practically the same during periods 55 and 56 as they were in period 54. When calcium chloride was omitted, period 60, the serum calcium fell to 9.0 mgm and the positive calcium balance was greatly reduced. The serum phosphorus rose to 4.6 mgm and the positive phosphorus balance was slightly increased. On resuming calcium chloride therapy, the serum calcium rose to 10.0 mgm and the positive calcium balance was greatly increased, whereas the serum phosphorus fell and the positive phosphorus balance was again decreased.

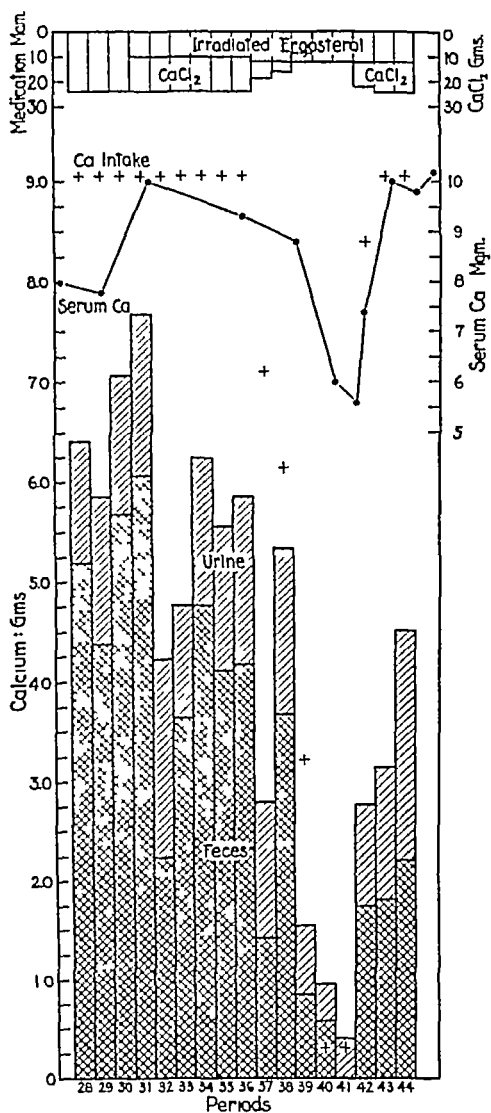
### *Experiment II*

Master A. P., an 8-year old schoolboy, was known to have had idiopathic parathyroid tetany since the age of 4. His dietary calcium intake was kept constant throughout the experiment, but was inadequate, 0.30 gram per three-day period. His data are presented in Charts 2A, 2B and Table I.

His total calcium intake during the control periods was brought up to an average of 9.05 grams per period by administering calcium chloride. The data obtained during the period of irradiated ergosterol medication were similar to those described in experiment I. (See Table I, periods 31-36.) Following the discontinuance of calcium chloride medication the calcium balance became negative and the positive phosphorus balance was slightly increased. The resumption of calcium chloride administration resulted in a positive calcium balance, much greater than that observed during comparable periods earlier in the experiment (periods 31-36).

The changes in the phosphorus metabolism were similar to those observed in the previous experiment.

In this experiment the changes in the serum calcium and phosphorus were very marked. During the control periods values of 8.0 and 7.8 mgm were obtained for the serum calcium and 6.25 and 7.41 mgm for the serum phosphorus. On the third day of ergosterol therapy, the serum calcium had risen to 10.0 mgm and the serum phosphorus had fallen to 7.14 mgm. With the discontinuance of calcium chloride during periods 40, 41, and 42, the serum calcium dropped to 5.6 mgm and the serum phosphorus rose to 9.2 mgm. On resumption of calcium chloride therapy, the serum calcium rose abruptly to 10.0 mgm and the serum phosphorus fell to 6.55 mgm. These values were maintained throughout the remainder of the experiment.



### CHART 2A CALCIUM METABOLISM IN EXPERIMENT II



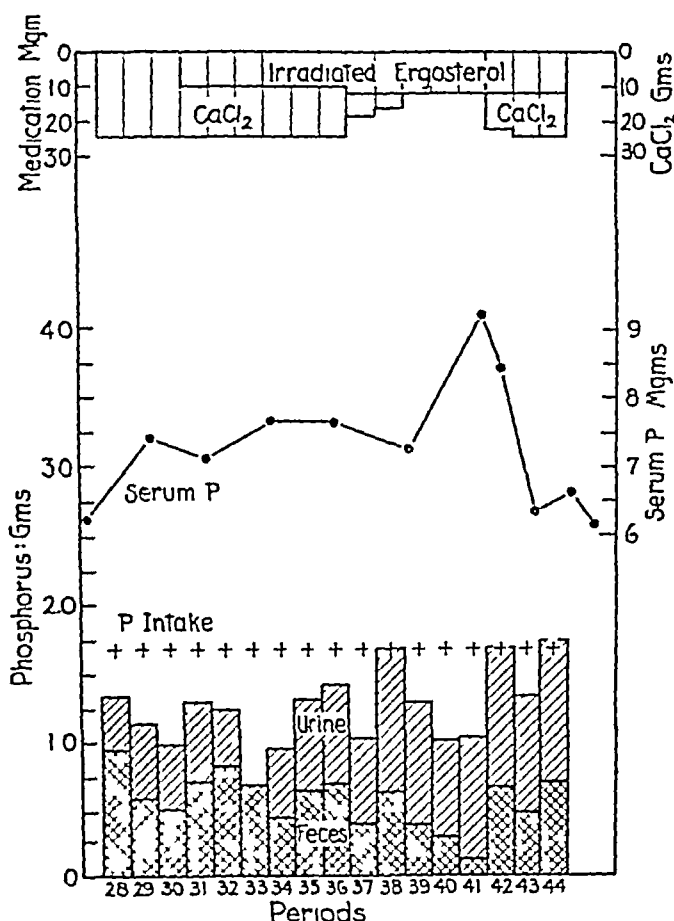


CHART 2B PHOSPHORUS METABOLISM IN EXPERIMENT II

*Experiment III*

Miss K R, a 26-year old school health worker, entered the hospital first on December 19, 1928. Subtotal thyroidectomy was done on December 31, 1928. Because of the persistence of symptoms of thyrotoxicosis a second operation was done on May 23, 1929, when more thyroid tissue was removed. Three days later, carpopedal spasms were noted. The serum calcium was found to be 6.8 mgm per 100 cc and the serum phosphorus 4.6 mgm per 100 cc. She was transferred to the Metabolism Ward for study and was placed on a low calcium diet (0.30 gram per three-day period). On June 13, the serum calcium had fallen to 5.2 mgm and the serum phosphorus had risen to 6.7 mgm. It is noteworthy that in the progression toward abnormal blood values the calcium was found low at the time when carpopedal spasms were first noted, whereas the phosphorus rose gradually to its high level some eleven days later. Charts 3A, 3B and Table I contain the calcium and phosphorus metabolism data from this patient.

From Table I one notes that the administration of irradiated ergosterol caused only slight changes in the calcium metabolism while this subject was on an inadequate calcium intake. The changes were so slight that one hesitates to ascribe them to irradiated ergosterol therapy

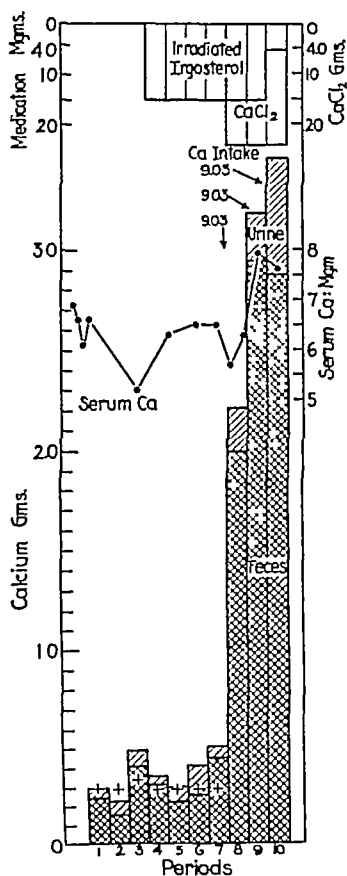


CHART 3A CALCIUM METABOLISM IN EXPERIMENT III

The urinary phosphorus rose, the fecal phosphorus remained practically unchanged, and the positive phosphorus balance decreased. During the last three periods of this study, the calcium intake was increased to 9.03 grams per period by the administration of calcium chloride. Irradiated

ergosterol therapy was continued during these three periods. A larger positive calcium balance ensued and the positive phosphorus balance decreased.

During the control periods the serum calcium varied between 5.2 and 6.6 mgm, the serum phosphorus rose from 5.44 to 6.72 mgm. During the administration of irradiated ergosterol the serum calcium remained almost constant, 6.3 to 6.5 mgm, the serum phosphorus gradually rose from

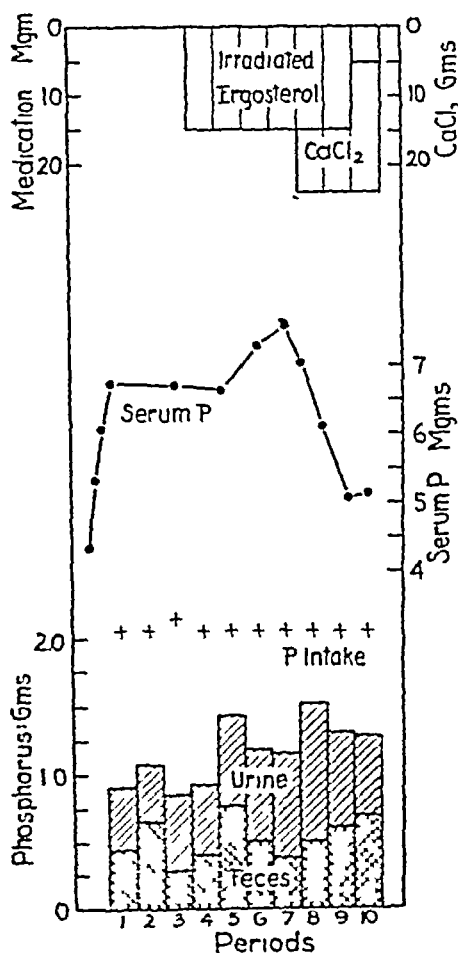


CHART 3B PHOSPHORUS METABOLISM IN EXPERIMENT III

6.7 to 7.6 mgm per 100 cc. When calcium chloride was administered in addition to the irradiated ergosterol, the serum calcium rose to 7.9 mgm (seventh day of therapy) and the serum phosphorus fell to 5.1 mgm. As our supply of irradiated ergosterol gave out on this day, none was administered during the last two days of period 10. This fact would seem to be an adequate reason for not having established normal serum calcium and phosphorus values in this case.

## DISCUSSION

From these experiments it is apparent that the beneficial action of irradiated ergosterol therapy on patients with parathyroid deficiency is dependent upon the calcium intake. The administration of 5 mgm of irradiated ergosterol per day in experiment III was without effect on the serum calcium or phosphorus or the calcium and phosphorus metabolism when the calcium intake was inadequate (0.30 gram per three-day period). When the calcium intake was increased to 9.03 grams per period, there resulted a marked retention of calcium and a rise in the serum calcium, an increased phosphorus excretion and a fall in the serum phosphorus. Failure to obtain a greater elevation in the serum calcium was very likely due to the fact that the irradiated ergosterol was administered for only seven days.

In experiment II, the calcium intake was high (9.05 grams per period) during the pre-medication periods, yet the serum calcium and phosphorus values were characteristic of those found in parathyroid tetany. When irradiated ergosterol was administered the positive calcium balance increased and the serum calcium rose, whereas the phosphorus excretion increased and the serum phosphorus fell. Reduction of the high calcium intake to an inadequate one by discontinuing the calcium chloride therapy caused the calcium balance to become negative, the serum calcium to fall, the phosphorus excretion to decrease, the serum phosphorus to rise, and as a consequence the signs and symptoms of tetany returned. With the resumption of the calcium chloride there occurred the following changes: a marked positive calcium balance, a rise in the serum calcium, an increased phosphorus excretion and a fall in the serum phosphorus.

The results of experiment I were similar to those of experiment II, except that with the discontinuance of the calcium chloride there was no return of the signs and symptoms of tetany and the drop in serum calcium and rise in serum phosphorus were not so marked. These differences can be accounted for by the fact that the dietary calcium intake in experiment I was adequate whereas in experiment II it was inadequate.

These observations on patients with parathyroid tetany show that if the calcium intake is inadequate the administration of irradiated ergosterol is without effect, yet its administration in the same dosage when the calcium intake is high results in complete symptomatic relief and changes in the calcium and phosphorus metabolism corresponding to those reported in Studies I and II (11-12). When irradiated ergosterol was administered to patients receiving a high calcium diet, the following changes occurred: a moderate decrease in the fecal calcium, a rise in the serum calcium, a slight rise in the urinary calcium, and a consequent increase in the positive calcium balance. However, the phosphorus excretion was increased and the serum phosphorus fell.

If the changes in the calcium and phosphorus metabolism observed in these patients are interpreted as being secondary to increased calcium absorption from the gastro-intestinal tract, they can be readily explained on the basis of the classification of disorders of calcium and phosphorus metabolism given by Albright, Bauer, Cockrill and Ellsworth (14). According to this classification, disorders of the parathyroid glands represent conditions in which the body fluids contain the saturating amount of calcium phosphate, but the proportion of the calcium ion to the phosphate ion is abnormal. In parathyroid tetany the calcium ion is reduced and the phosphate ion is increased. Because the body fluids already contain a saturating amount of calcium phosphate, the rise in the serum calcium (due to the increased absorption of calcium from the gastro-intestinal tract) is accompanied by a fall in the serum phosphorus and an increased phosphorus excretion in an attempt to keep the  $\text{Ca} \times \text{P}$  product normal. However, the fall in the serum phosphorus is not sufficiently rapid and therefore the  $\text{Ca} \times \text{P}$  product rises.

Further evidence to substantiate the belief that the beneficial action of irradiated ergosterol in parathyroid tetany is directly related to the calcium intake and is not secondary to the stimulation of any remaining parathyroid tissue is obtained when one compares the changes in the calcium and phosphorus metabolism of a normal individual while receiving an inadequate calcium intake plus an active parathyroid extract and when he is on the same diet but receiving irradiated ergosterol instead of parathormone.<sup>5</sup>

With the administration of an active parathyroid extract to a normal individual on an inadequate calcium intake there occurs (15)

- 1 An increased urinary phosphorus excretion,
- 2 A fall in the serum phosphorus,
- 3 A rise in the serum calcium,
- 4 An increased urinary calcium excretion,
- 5 No effect on the fecal calcium or phosphorus excretion,

whereas the administration of irradiated ergosterol in adequate doses to a normal individual on a low calcium diet causes

- 1 A decrease in the fecal calcium,
- 2 A rise in the urinary calcium,
- 3 A decrease in the fecal phosphorus,
- 4 A rise in the urinary phosphorus,
- 5 Only slight elevation of serum calcium,
- 6 Little, if any, elevation of serum phosphorus

Thus, the action of the two agents which are of most importance in the regulation of normal calcium phosphate metabolism is exactly op-

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<sup>5</sup> The trade name for the active principle of the parathyroid glands as prepared by the Eli Lilly Company

posite, the active principle of the parathyroid glands mobilizing calcium and phosphorus from the bones, vitamin D increasing absorption of these elements from the gastro-intestinal tract

All the data presented in this series of papers strengthen the theory which has long been held by certain workers (16-27) Various workers have reported a shift in the pH of the stools from alkaline to acid when individuals with rickets are receiving vitamin D An increased acidity of the gastro intestinal tract would favor more complete absorption of calcium and phosphorus (28-29), yet analysis of the gastric and pancreatic juices has failed to reveal any increase in acidity during irradiated ergosterol therapy (35) Therefore, we are left with no explanation of the increased absorption of calcium and phosphorus from the gastro-intestinal tract or of the reason for the observed shift in the pH of the stools The increased acidity of the stools in rickets may be secondary to other changes such as (1) the secretion of a more acid succus entericus or (2) alteration of the permeability of the intestinal wall, thereby allowing more complete absorption of calcium and other basic ions Further study is necessary to prove either of these last two mentioned modes of action

Overdosage of irradiated ergosterol results in mobilization of calcium phosphate from the bones and decalcification (30) Because of this mobilization of calcium phosphate, the body fluids contain more than the normal amount of calcium phosphate, and calcium phosphate deposition takes place in tissues other than bones This metastatic calcification is found more commonly in tissues where the carbon dioxide tension is more likely to be low (31) Why therapeutic doses of irradiated ergosterol result in an increased absorption of calcium and phosphorus from the gastro intestinal tract and overdosage in mobilization of calcium phosphate from the bones cannot be answered accurately at this time, but there is an increasing amount of evidence which suggests that the toxic effects from excessive irradiated ergosterol dosage are due to contained impurities rather than to the vitamin D concentrate itself (32-34)

From these experiments as well as from those previously published it is apparent that small doses of irradiated ergosterol produce beneficial effects on patients with disorders of calcium and phosphorus metabolism, but the same doses are without effect on the calcium and phosphorus metabolism of normal individuals These results further demonstrate that the dose required for adults is larger than that usually prescribed for infants and children Therefore, it would be well to have this potent vitamin D concentrate prepared in two different strengths, one for children and one for adults It must be remembered that the preparation used in these studies contained 10 mgm in each cc. and that 0001 to 00025 mgm was sufficient to protect against rickets in rats fed on the Steenbock diet

## SUMMARY

1 The beneficial results obtained in the treatment of parathyroid tetany when therapeutic doses of irradiated ergosterol are employed are directly related to the calcium intake and are not secondary to the stimulation of remaining parathyroid tissue

2 The administration of 5 mgm of irradiated ergosterol a day to an individual with parathyroid tetany on an inadequate calcium intake is without beneficial effects

3 The administration of 5 mgm of irradiated ergosterol a day to an individual with parathyroid tetany receiving a high calcium intake produces the following changes

- a* A decreased fecal calcium excretion,
- b* An increased urinary calcium excretion,
- c* An increased positive calcium balance,
- d* A rise in the serum calcium,
- e* An increased fecal phosphorus excretion,
- f* An increased urinary phosphorus excretion,
- g* A decrease in the positive phosphorus balance,
- h* A fall in the serum phosphorus,
- i* The serum calcium rises faster than the serum phosphorus falls and therefore the  $\text{Ca} \times \text{P}$  product rises,
- j* The signs and symptoms of tetany disappear

4 The dose of irradiated ergosterol required to produce beneficial changes in the calcium and phosphorus metabolism of individuals with calcium and phosphorus disorders is smaller than the amount necessary to produce similar changes in the calcium and phosphorus metabolism of normal individuals

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# THE MOVEMENT OF FLUID THROUGH THE HUMAN CAPILLARY WALL IN RELATION TO VENOUS PRESSURE AND TO THE COLLOID OSMOTIC PRESSURE OF THE BLOOD

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It is well established by experiments on normal human subjects that when venous pressure is elevated, fluid is filtered from the blood into the tissue spaces. This filtration has been identified by the increase in the volume of the congested limb (Mende, 1919, Carrier and Rehberg, 1923, Drury and Jones, 1927), and also by the concentration of protein in the blood which has passed through the congested limb (Rowe, 1916). There is no information, however, concerning the effect in normal human subjects of changing the other equally important factor, namely, the colloid osmotic pressure of the blood.

The association of a low colloid osmotic pressure of the blood with certain forms of edema occurring as a result of disease in man (Krogh, 1922, Hagedorn, Rasmussen and Rehberg, 1922, Govaerts, 1924, Schade and Claussen, 1924, and others), or occurring with experimental hypoproteinemia in dogs (Leiter, 1928) suggests, but does not prove, that the movement of fluid through the capillary wall is related to the colloid osmotic pressure of the blood. The further finding that capillary pressures in the frog, certain rodents, and man (Landis, 1926, 1930) are approximately equivalent to their different blood colloid osmotic pressures is also suggestive, but not conclusive, evidence that the Starling conception probably underlies the mechanism of fluid balance in the mammal.

If fluid balance in man depends upon an approximate balance between capillary pressure and the colloid osmotic pressure of the blood two things must follow. In the first place, as mentioned by Krogh (1929), a relatively small elevation of venous pressure must suffice to cause fluid to accumulate in the tissue spaces, so that the erect human being is constantly near to edema. In the second place, with any given capillary pressure, a rise in the colloid osmotic pressure of the blood must be accompanied by a fall in the rate of filtration.

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These two points have been investigated in normal human subjects by two plethysmographic methods of determining arm volume. The results indicate that fluid begins to accumulate in the tissue spaces at venous pressures of 17 cm water or more, and that at a given venous pressure a rise of the colloid osmotic pressure of the blood is associated with a decrease in the rate at which fluid filters through the capillary wall.

*The measurement of fluid filtration by means of the ordinary plethysmograph*

The relation between venous pressure and the rate at which fluid is filtered through the capillary wall was first studied by means of an adaptation of the plethysmograph described by Lewis and Grant (1925). The method and the results obtained with it will be described only in briefest outline since, in spite of careful selection of subjects, it was found that spontaneous variations in arm volume precluded the use of this method for quantitative measurements of the very small amounts of fluid accumulating in the tissues during short periods of slight or moderate venous congestion. The results, inconstant though they are, merit a brief description since they provide qualitative support to certain conclusions reached by another method.

The subjects, 3 women and 1 man, were healthy adults with normal blood pressure, selected from a group of 19 because they showed the smallest spontaneous changes in arm volume when plethysmographic records were made over a period of an hour or more. During an observation the subject reclined with the elbow and hand supported and fixed by sandbags so that the upper surface of the forearm was level with the manubrium sterni. On each forearm was placed a plethysmograph 15 cm long, the ends of which were closed by means of rubber diaphragms which were accurately fitted to the arm to form an airtight joint without raising venous pressure above 10 cm water (Fig 1, *a*). Each plethysmograph was connected with a small spirometer of the Krogh type, having a total capacity of about 40 cc. The whole system was filled with air, and the variations in arm volume were recorded on a slowly moving kymograph by two pens, one writing above the other (Fig 1). In these records pulse and respiratory waves were barely visible, and did not interfere with the measurement of the volume changes.

In each experiment one arm was used as a control (lower lines, Fig 1 *a, b, c*) and, after a rest period of 30 minutes or more, venous pressure in the other arm was raised to 15, 20, 25, 30 or 40 cm water for 15 minutes by means of a Riva Rocci armlet, 15 cm wide. Figure 1 shows records of the volume changes produced by 15 cm water (Fig 1, *a*), 40 cm water (Fig 1, *b*) and 20 cm water (Fig 1, *c*), the last showing also moderately large spontaneous variations in volume even during the period of congestion. The greatest increase in volume, due to filling of veins and distention of minute vessels, occurred before the fifth minute. From the fifth

to the fifteenth minute the line was approximately straight with a slope depending on the degree of venous congestion

In such a closed system containing air it was essential that temperature be carefully controlled. This was done in the first place by using one arm as a control, and by measuring the volume change in terms of differ-

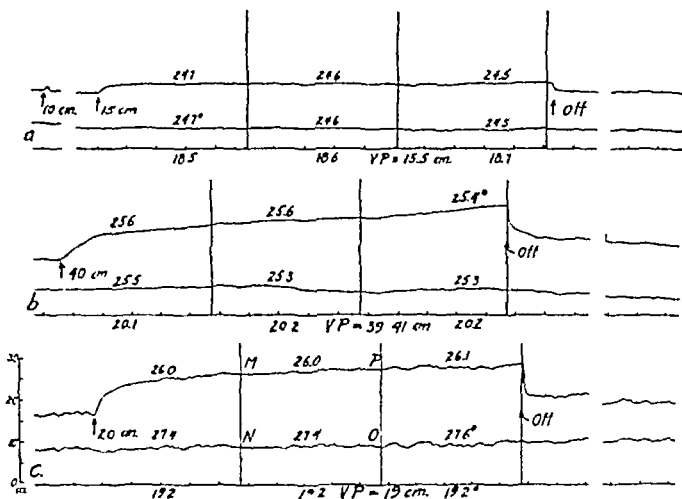


FIG 1 RECORDS OF CHANGE IN ARM VOLUME DURING 15 MINUTES WITH VENOUS PRESSURES OF (a) 15 CM, (b) 40 CM, AND (c) 20 CM WATER

In each record the upper line shows the volume change in the experimental arm, the lower line the volume change in the control arm. The temperature of the air in the plethysmograph is indicated at intervals on each record. Room temperature is recorded under the time line. To the left in (a) is shown the increase in volume when venous pressure was raised for a few seconds to 10 cm water, indicating that venous pressure had previously been less than 10 cm water. On the right is shown the volume 15 minutes after the release of congestion.

*Note.* The records, originally written in red, were traced over with black ink to permit marked reduction without loss of clearness. Time, originally marked in 1 minute intervals, is shown only in 1 minute intervals in this figure. To the left is given a composite volume scale made by calibration of both spirometers.

ence between the two records. The temperature of the air within the plethysmograph changed equally in the control and the experimental arm, usually in the downward direction, by an average of 35° C in an experimental period of 30 minutes. Room temperature varied usually only 1 to 2° C, very rarely more than 3° in the same period. The skin

temperature of the segment of arm enclosed within the plethysmograph was about  $31\text{--}32^{\circ}\text{C}$ , while the air in the plethysmograph had a temperature between  $24.0$  and  $28.0^{\circ}\text{C}$ . Room temperature was between  $18$  and  $20^{\circ}$  except in four instances when it was between  $20$  and  $21^{\circ}\text{C}$ . Careful examination of the results indicated that the variability in the findings could not be explained on the basis of temperature changes.

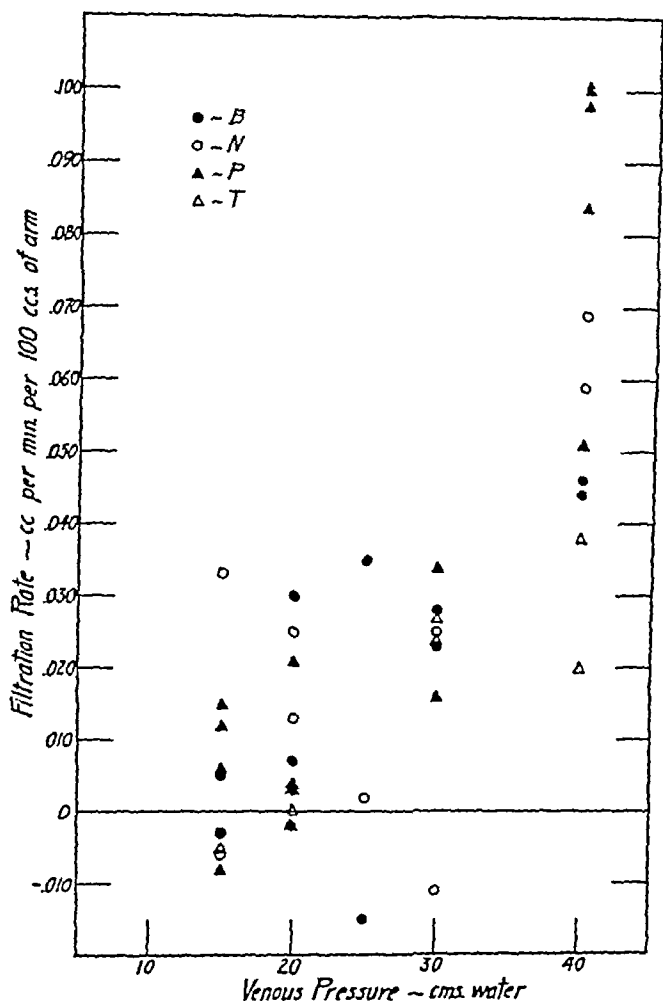


FIG 2 RELATION BETWEEN FILTRATION RATE (CC PER MINUTE PER 100 CC OF ARM) AND VENOUS PRESSURE (CM WATER) IN FOUR SUBJECTS, AS DETERMINED WITH THE ORDINARY PLETHYSMOGRAPH

The computation of filtration rates was frequently complicated by spontaneous variations in volume (Fig 1, c) which prevented the use of ordinary methods of measurement. Therefore lines were drawn vertically in each record from the time line at the 5th, 10th and 15th minutes. The area *MNOP* (Fig 1, c) was measured by planimeter and the figure thus obtained was divided by the linear distance *NO* to determine the average

(591)

distance in millimeters between the two curves during the period from the fifth to the tenth minutes. The same procedure was used to determine the average distance between the two curves during the period from the tenth to the fifteenth minutes. The difference between these distances, when compared with the calibration figures of the spirometers, indicated the change in volume of the experimental arm compared to the control arm during five minutes' congestion. The volume of the segment of arm within the plethysmograph was measured (as described on page 73) and the apparent rates of fluid movement were computed in terms of cc of fluid per minute per 100 cc arm. The results have been charted in Fig 2 to show the relation between the apparent rates of filtration and venous pressure.

The accuracy of this method of measuring the rate at which fluid is filtered into the tissue spaces depends obviously upon the quantitative identity of the spontaneous volume variations in the two arms. Figure 2 shows by the large spread, in sign as well as in magnitude, of the values thus obtained, that the spontaneous variations of volume are not entirely identical in the two arms. This conclusion was verified also by examination of single experiments (Fig 1, c). From this it appears evident that a simple plethysmograph is unsuited for the accurate measurement of small amounts of fluid accumulating in the arm at relatively low venous pressures, since the unavoidable vasomotor variations produce spontaneous changes in arm volume which are often greater than the volumes of fluid to be measured (Fig 1, c).

The results are of value in that they indicate vaguely what was verified definitely by another method. Filtration of fluid may or may not result when venous pressure is elevated to 15 or 20 cm water, but filtration is clearly evident at a venous pressure of 30 cm water, becoming still greater at a venous pressure of 40 cm water.

### *The pressure plethysmograph*

Quantitative studies of filtration were made by a method which determined the change in volume of the tissues and their contained extravascular fluid, while excluding the contents of the blood vessels. For this purpose pressure was exerted on the surface of the segment of arm within the plethysmograph, to collapse the blood vessels before the final volume was measured. Under these conditions the state of contraction or dilatation of the blood vessels did not, within certain limits, interfere with the reasonably accurate measurement of changes in the volume of tissue fluid.

(a) *The apparatus* The plethysmograph, shown in Fig 3, was made of sheet metal (*P*), 5 mm in thickness, in the form of a truncated cone, having a length of 15 cm. Two sizes were used, the smaller with end diameters of 9 and 10 cm, the larger with end diameters of 10 and 11 cm. A sleeve (*S*) of thin rubber (25 mm thick), 33 cm long, 9 and 10 cm in

diameter at the ends, was placed inside the metal case and the ends were everted and attached firmly ( $S'$ ) to the ends of the plethysmograph, by means of cement to ensure a water tight junction. When collapsed the folds of the inner bag extended about 3 cm beyond the ends of the metal sleeve, and when filled with water under pressure, the surplus rubber lay against the surface of the arm in a series of folds ( $F$ ). The ends of the plethysmograph were closed by diaphragms ( $D$ ) of heavier rubber, 1.0 mm in thickness. Each diaphragm was cut from rubber obtained in the form of a truncated cone 15 cm long, 5 and 9.5 cm in diameter at the ends. The segments were accurately fitted to the arm so that the diaphragms, while tightly stretched over the ends of the metal sleeve, were in gentle contact with the skin of the arm over a distance of 1.5 to 2.0 cm

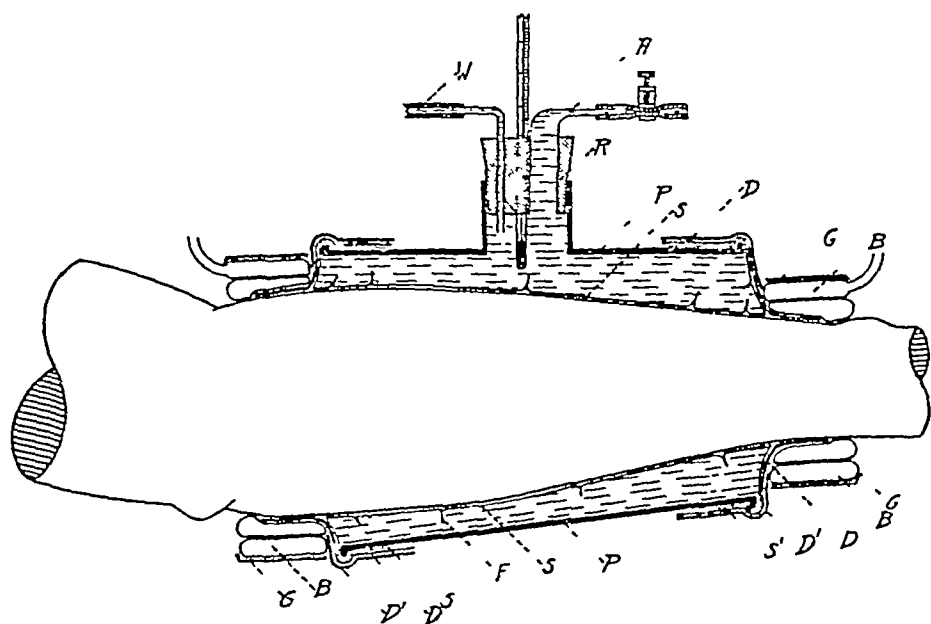


FIG 3 DIAGRAM OF THE PRESSURE PLETHYSMOGRAPH  
For description see text

To make the ends less distensible a second partial diaphragm of rubber ( $D'$ ) was arranged to extend half way between the metal sleeve and the skin.

Two rubber bags, 60 cm long and 3.5 cm wide, were wound ( $B$ ) 2 or 3 times around the arm above and below the plethysmograph over the skin and the outer portion of the diaphragms. Each bag was held against the arm by several loose turns of gauze bandage ( $G$ ) so that the inner surface of the rubber bag was in immediate contact with both the diaphragms and the skin. Both bags were connected with a compressed air supply of 55 cm water pressure, so that, before the pressure inside the plethysmograph was raised, they might be inflated to prevent the thin inner bag from being pushed out between the diaphragms and the skin.

The large opening in the top of the plethysmograph was closed by a rubber stopper (*R*) which held one large glass tube (*A*) to catch any bubbles of air left within the bag, and one small glass tube (*W*) through which water moved from the plethysmograph to the burette in which volume change was measured. A thermometer inserted into the stopper indicated the temperature of the water surrounding the arm. The movement of 40 to 60 cc of water into and out of the plethysmograph during each volume determination provided adequate mixing. Temperature was measured in a few observations by 2 thermal junctions, one above and the other below the arm, without detecting any significant error in the simpler method. The metal surface of the plethysmograph was covered with cotton wool to diminish heat loss.

The plethysmograph was connected, by pressure tubing running from *W* (Fig 3), to the lower end of a 100 cc burette with 2 cc. graduations. The upper end of the burette was connected to a compressed air supply by means of which a pressure of 55 cm water could be exerted on the contents of the burette, and through *W* to the inside of the plethysmograph.

Venous congestion was produced by inflating a cuff, 50 cm long and 15 cm wide, which encircled the upper arm just above the elbow. To obstruct arterial inflow to the arm (except through the bone) a second cuff, 7 cm wide and 55 cm long, was placed just below the axilla. This latter cuff was inflated rapidly from a reservoir to a pressure of 240 mm Hg or more. Venous pressure was determined in the veins of the hand both before and during venous congestion by a capsular method described elsewhere (Krogh, Turner and Landis, 1932).

The subjects in all but one series of observations were recumbent with the hand and elbow placed on sandbags so that the plethysmograph was supported only by the forearm. The upper surface of the forearm was level with the manubrium sterni, the arm was abducted to about 30° at the shoulder, and the elbow was slightly flexed. To avoid movement and the resulting changes in the relation between the arm and the plethysmograph the distal portion of the hand was weighted with sandbags, leaving on the dorsum of the hand only enough room for the venous pressure capsule. It was essential that the subject be absolutely still if reliable readings were to be obtained, and every effort was made to have the subject entirely comfortable before an experiment was begun. The observations in this series were made exclusively on two subjects, one of whom (E L) habitually showed large spontaneous changes in arm volume when measured by the ordinary plethysmograph. Blood pressure in both subjects was within the normal range.

At the beginning of an experiment the inner bag of the plethysmograph was arranged so that the extra folds of rubber were collected at the ends of the plethysmograph. The instrument was then slipped on



the forearm until it reached the position shown in Fig 3. A short piece of rubber pressure tubing, 4 mm in outside diameter, with many perforations along its length, was placed between the lateral surface of the forearm and the inner bag, so that it ran completely through the plethysmograph while its ends projected beyond the two diaphragms. Thus any air caught between the inner bag and the skin could escape by this channel when water flowed into the plethysmograph.

To keep the plethysmograph from slipping down the forearm toward the wrist the proximal rubber diaphragm was attached to the skin distal to the elbow joint by means of several short pieces of wide surgeon's plaster. The two external rubber bags were then wrapped loosely about the diaphragms above and below the plethysmograph as shown in Fig 3.

Water at a temperature of 34° C was poured into the burette and from there entered through *W* into the inner bag of the plethysmograph, which gradually filled the space around the arm and finally pushed the thin rubber in folds against the skin and the diaphragms. As the water distended the bag the plethysmograph was shaken vigorously to dislodge the air not only from the inner surface of the bag but also from the space between the bag and the skin. The former collection of air was removed through *A* (Fig 3), the latter collection found its way under the diaphragms along the rubber tube, which was left between the inner bag and the skin throughout the experiment. The burette was filled approximately to the 20 cc mark and the top of the burette was connected with a tube leading to the compressed air supply.

The forearm is conical in shape and when the pressure in the plethysmograph was raised the whole apparatus tended to slip downward until it was retained by the adhesive tape connecting the upper diaphragm and the skin. In order to settle the apparatus in this final position as rapidly as possible the pressure in the plethysmograph was raised and lowered rapidly and the circulation was cut off several times before the first determination of volume. This diminished the time required for obtaining the series of constant readings which must necessarily precede the measurement of the volume change produced by experimental procedures.

The surface of the water in the burette was always kept level with the upper surface of the plethysmograph. The amount of water in the burette-plethysmograph system was the same through each experiment, so that a decrease in the volume of the arm caused the surface of the water in the burette to fall below the level chosen as the point of reference. The burette was then raised to restore the water to its original level and, pressure being thus constant, the volume change could be read by comparing the volumes indicated on the burette. With a fall in arm volume therefore the burette reading became higher, with a rise in arm volume the burette reading became lower, by an amount equal to the change in arm volume. With normal circulation the spontaneous changes in arm vol-

ume amounted usually to between 1 and 3 cc, comparing favorably with the spontaneous variations observed with the ordinary plethysmograph

Venous pressure was measured at this time and was ordinarily between 12 and 14 cm water in the hand below the plethysmograph. If the venous pressure was over 15 cm water the diaphragms and cuffs were refitted and adjusted until a venous pressure less than 15 cm was observed, in order to be certain the filtration was not produced by congestion due to the plethysmograph itself

(b) *The determination of "reduced arm volume"* The term "reduced arm volume" will be used to designate the volume of the segment of arm in the plethysmograph when the blood vessels were collapsed by the external pressure. "Arm volume" will refer to the volume of the arm segment when the blood vessels were filled. When the pressure was released between the determinations of "reduced arm volume" the apparatus recorded changes in "arm volume" as an ordinary plethysmograph would have done

In each determination of reduced arm volume the two small external bags were first inflated with a pressure of 55 cm water (A, Fig 4). From 3 to 5 seconds later (B, Fig 4) the same pressure was applied to the contents of the burette. Water left the burette rapidly, but the burette was raised in its holder so that the surface of the water was always kept level with the upper surface of the plethysmograph. From 30 to 45 cc. of water left the burette in the first 10 to 15 seconds, due in part to removal of blood from the arm and in part to bulging of the diaphragms under the internal pressure. Circulation was not stopped at this time in order that the greater part of the blood expressed from the forearm might be carried away through the open veins. The brachial artery and vein were compressed about 25 seconds (C, Fig 4) after the inflation of the external bags, by raising the pressure in the upper, narrow armlet to 240 mm Hg or more. The residual blood could then be expressed from all the blood vessels within the plethysmograph, including arteries and arterioles, by the external pressure of 55 cm water. This relatively small volume of blood was accommodated by the veins situated in the segment of the arm between the lower border of the occluding armlet and the upper border of the plethysmograph.

The pressure applied to the surface of the segment of arm within the plethysmograph must be high enough to express almost all of the blood within a few minutes, and yet be as low as possible to avoid expressing tissue fluid from the same segment of arm. It was found that 55 cm of water was the most suitable pressure in this regard since it removed the blood in the course of 3 to 6 minutes.

With this pressure, as shown in Fig 4, the change in volume in the first minute usually exceeded 40 cc, but in the second minute was reduced to between 2 and 4 cc, depending on the size of the arm and, as will be

shown later, on the state of the vessels in the arm. After the second minute, readings of volume were made every 30 seconds, the change in volume in each period becoming steadily less until after the 4th or 5th minute it was only 2 to 15 cc per 30 seconds. The volume continued to decrease quite constantly at this rate even when readings were continued for a total of 10 minutes.

In the usual determinations the readings were continued, as shown in Fig 4, until in each of 2 successive 30 second periods the volume changed 2 cc or less. This point was reached after the circulation had been stopped for a period of 3.5 to 6 minutes, or, more rarely with a larger arm,

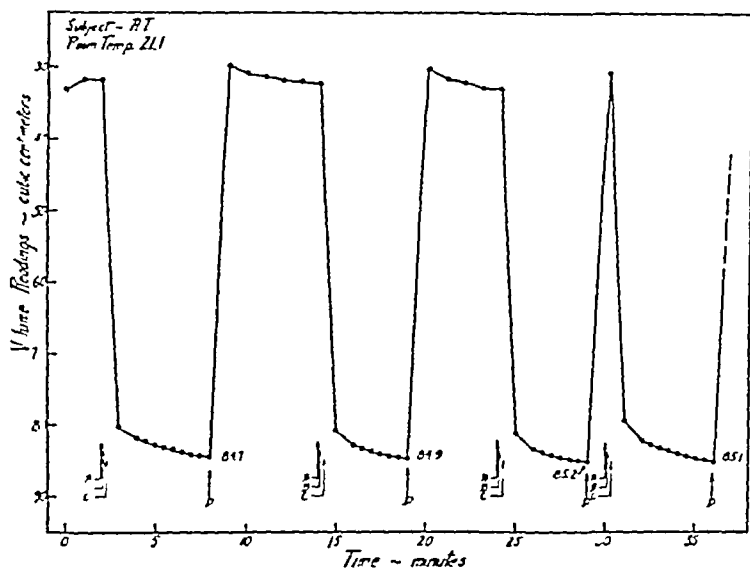


FIG 4 CHART SHOWING BURETTE READINGS BETWEEN AND DURING DETERMINATIONS OF REDUCED ARM VOLUME BY MEANS OF THE PRESSURE PLETHYSMOGRAPH

The first three determinations were made during normal resting circulation. The last determination was begun during the period of reactive hyperemia which followed the last control estimation. For full description see text.

7 minutes. Readings were never continued beyond 7 minutes because an excessively long anoxemia might modify the permeability of the capillary wall. There was some indication that this occurred if circulation was cut off for 10 minutes or longer, a period of 7 minutes was therefore regarded as the extreme limit of complete safety.

The reading obtained at the end of one minute of minimal change in volume (2 cc per 30 seconds) was recorded for comparison with other readings made under the same conditions in later determinations. In Fig 4 the burette reading at the end of the first determination was 84.7 cc. Pressure was released (D, Fig 4) first from the inner bag, a few seconds later from the external bags, and finally about 10 to 15 seconds later from the armlet. Circulation was resumed, and, due to reactive hyper-

emia, the arm volume one minute later was 2.5 cc higher than at the beginning of the determination.

A period at least as long as the preceding occlusion was allowed to elapse before the next determination, this seemed sufficient since Lewis and Grant (1925) found that reactive hyperemia lasted not more than  $3/4$  as long as the occlusion period which produced it. A second determination made in the experiment shown in Fig. 4 yielded a final reading of 85.0 cc., and a third, after a similar recovery period, 85.2 cc. Reduced arm volume therefore varied in these three control readings through a range of .5 cc.

At the beginning of each experiment determinations were made in this way at intervals of 10 to 15 minutes until 3 consecutive readings were obtained with a total variation of .5 cc. or less. This occurred frequently as in Fig. 4 in the first 3 determinations, but more often the first 2 or 3 readings showed a greater variation usually in the direction of an apparent decrease in arm volume. This may have been due to the removal of small amounts of tissue fluid from the arm, or to slight change in the relation between the plethysmograph and the arm. The final agreement, however, was at times as close as 1 to 3 cc., with variations lying in a haphazard way both above and below the average value, as shown in the control period in Fig. 5. In the experiments where total variation could not be reduced to less than .5 cc. after a number of trials the observations were discontinued, usually with the discovery of a hidden leak in the inner bag, or of an unequal distribution of the folds in the inner bag.

Having obtained three control readings within the limits mentioned above, venous congestion was applied for a given period of time. One minute after the release of the congestion reduced arm volume was determined again. The difference between this last reading and the average of the 3 control readings indicated the volume of "tissue fluid" which had accumulated during the period of congestion and still remained in the tissues. It is likely that the lymph vessels were emptied by the external pressure in the same way as the blood vessels, so that the increase in reduced arm volume represented the volume of "tissue fluid."

At the end of the experiment the apparatus was removed and the approximate volume of the segment of arm enclosed by the plethysmograph was determined. The upper and lower boundaries of the inner bag were clearly marked on the skin since the folds of rubber produced elevations in the skin not unlike minute linear wheals. The circumference of the arm was measured at both boundaries and from the average circumference and the length of the arm segment the volume could be computed. This method of measurement was only approximate but nevertheless agreed fairly well with determinations of volume made by displacement of water. Moreover examination of the data below will show that other unavoidable variations made a more exact measurement unnecessary. In sub-

ject A T the volume of forearm within the plethysmograph was between 445 and 540 cc, in subject E L between 580 and 690 cc, the exact figure depending upon the nearness of the plethysmograph to the elbow

The rate of filtration at a given venous pressure was computed by dividing the increase in reduced arm volume by the duration of the congestion and by the volume of the arm segment. The filtration rates are expressed in terms of cc of fluid filtered per minute per 100 cc of arm

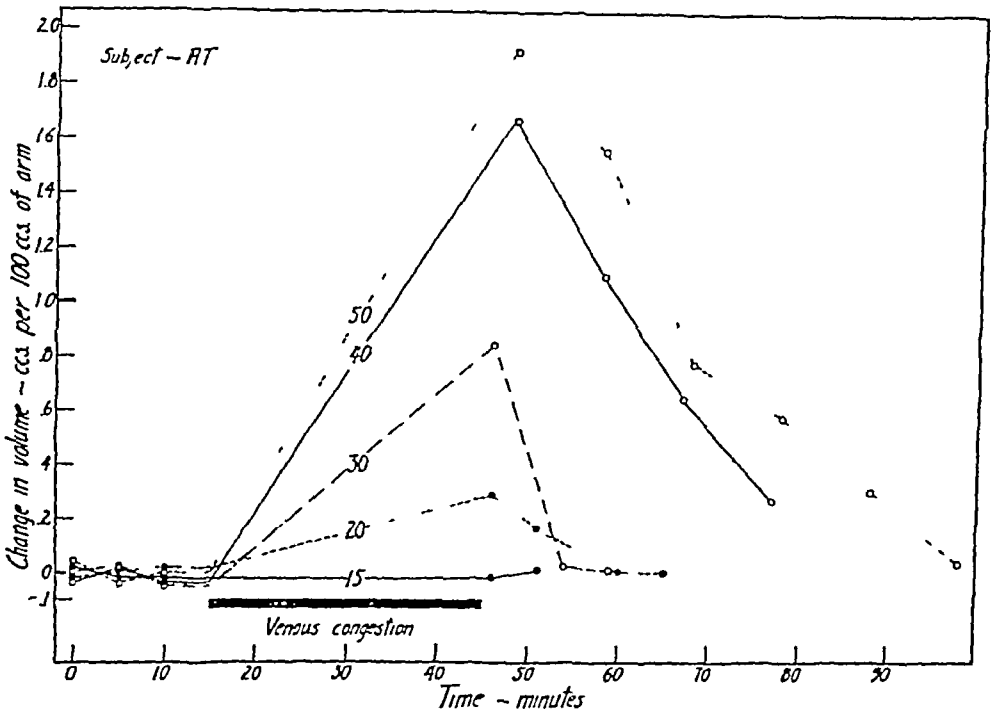


FIG 5 CHART SHOWING THE ACCUMULATION OF TISSUE FLUID (CC PER 100 CC OF ARM) PRODUCED BY VENOUS CONGESTION OF 15, 20, 30, 40 AND 50 CM WATER PRESSURE APPLIED OVER A PERIOD OF 30 MINUTES

To the right is shown the removal of the tissue fluid after release of the venous congestion

Each dot or circle represents a determination of reduced arm volume, requiring from 4 to 5 minutes' stoppage of circulation

Where volume changes alone are mentioned they are recorded in terms of cc of fluid per 100 cc of arm

The further details of method apply only to single groups of experiments and will be taken up under the appropriate headings

#### Observations

(a) *Hyperemia and reduced arm volume* The usefulness of the pressure plethysmograph depended upon the extent to which the reduced arm volumes were independent of vascular tone. This was tested by comparing the reduced arm volumes determined during normal circulation with those determined during reactive hyperemia. Figure 4 shows

one of these experiments with 3 control determinations of reduced arm volume, each begun during normal circulation, and a fourth determination which was begun in the middle of the reactive hyperemia which followed the last control observations. Arm volume, due to vascular dilatation, was 2.4 cc. higher, but reduced arm volume was within 0.1 cc. of the last control reading.

In a group of six similar experiments arm volume was from 1.3 to 7.0 cc. greater in the hyperemic period which followed the complete occlusion of circulation for 5 to 10 minutes. Reduced arm volumes were unchanged or slightly less in 3 instances, and greater by only 0.1, 0.3 and 0.4 cc. in the other three. It may be noted also in Fig. 4 that when the determination of reduced arm volume was made during hyperemia the change in volume during the first few minutes was greater, and the time required for the volume change to reach the rate of 2 cc. per 30 seconds was longer. This was to be expected since a greater mass of blood must be expressed when the vessels are dilated.

Since the reduced arm volume was independent of vascular dilatation of the magnitude described above, it seemed safe to assume that the ordinary spontaneous changes in vascular tone had no significant effect on reduced arm volume. This, in fact, was frequently observed in single experiments, arm volumes often varied through a range of 3 cc. or more during the period when control readings were made, while the reduced volumes differed only by 1 to 3 cc.

(b) *The relation between filtration rate and venous pressure.* The minimal venous pressure required to produce filtration, and the change in the filtration rate with various degrees of venous congestion were studied in both subjects. Room temperature was between 20.0° and 21.5° C with the exception of one experiment in which it was 18.1°. The temperature of the water in the plethysmograph was between 26.8° and 30.5° C.

In the preliminary control period (Fig. 5, Protocol 1) venous pressure was determined, and three or more readings of volume were made in order to demonstrate that arm volume was not changing significantly. Five minutes after the end of the last control reading the wide armlet was inflated to the desired pressure. The effectiveness of the lesser degrees of congestion (12.5 to 30 cm. water) was verified by measuring venous pressure in the hand (Protocol 1). The venous congestion was continued for 30 minutes and then released. One minute was allowed for the escape of the blood temporarily accumulated in the arm by the congestion, as shown by the ordinary plethysmograph records (Fig. 1) this escape is practically complete in 45 seconds or less. At the end of this minute a determination of volume was begun, and later at intervals the determinations were repeated to follow the subsequent removal of the fluid.

Figure 5 shows the change in reduced arm volume, expressed in cc. per 100 cc. of arm, shown by subject A. T. during and after a 30 minute

February 25, 1931 Subject, A T, recumbent Plethysmograph on right arm

Time	Burette readings	Temperature of plethysmograph	Notes	Time	Burette readings	Temperature of plethysmograph	Notes
minutes 5-10	cc	° C		minutes	cc	° C	
10	18 0		Preliminary adjustments of plethysmograph	54	16 6	28 1	Venous pressure 14 5 cm
11	15 0		Venous pressure less than 15 cm water	55	16 9		20 cm pressure on wide armlet
12	15 0						
13	11 0			60	10 0	28 0	Venous pressure 20 0 cm
15	13 8	28 2	Pressures on	65	9 2	28 0	19 0
16	68 0			70	9 8	27 8	Room temperature 20 2° C
17	70 0			75	10 0	27 8	Venous pressure 20 0 cm
17 5	70 3			80	9 0	27 8	19 5
18	70 7			85	7 4	27 8	19 0
18 5	71 1						Room temperature 20 8° C
19	71 6						20 cm pressure off
19 5	71 8			86	15 5		Pressures on
20	72 0		Pressures off				
21	11 2	28 8	Venous pressure 23 cm	87	66 4		
22			18	88	68 8		
23			15 5	88 5	69 4		
24			15 to 14	89	69 7		Room temperature 20 8° C
25	11 0		Pressures on	89 5	70 1		
				90	70 3		Pressures off
				90 5	70 5		
26	68 1			91	14 6		
27	70 5			96	16 0		Pressures on
27 5	71 0						
28	71 1			97	66 8		
28 5	71 7			98	69 4		
29	71 9		Pressures off	98 5	70 0		
29 5	72 1			99	70 5		
				99 5	70 8		
				100	71 0		
				100 5	71 1		Pressures off

## PROTOCOL 1—Continued

Time	Burette readings	Temperature of piezograph	Notes	Time	Burette readings	Temperature of piezograph	Notes
<i>minutes</i>	<i>cc</i>	<i>C</i>		<i>minutes</i>	<i>cc</i>	<i>C</i>	
34	16.4		Venous pressure 14 cm	105	16.8	28.0	Venous pressure 14 cm
35			Pressures on	109	17.4		Pressures on
				110	17.4		
36	68.2			111	68.0		
37	70.4			112	70.5		
37.5	71.0			112.5	71.2		
38	71.4			113	71.6		
38.5	71.7		Room temperature 20.9° C	113.5	71.8		Pressures off
39	71.9		Pressures off	114	71.9		
39.5	72.0						
44				119	17.6		Pressures on
45	14.0	28.2	Venous pressure 15 to 11.5 cm	120	17.4		
			Pressures on				
46	67.2			121	68.4		
47	70.2			122	70.6		
47.5	70.8			122.5	71.2		
48	71.2			123	71.6		
48.5	71.5			123.5	71.8		
49	71.7			124	72.0		Observation ended
49.5	71.9		Pressures off				

Summary of experiment		
Time	Burette reading for reduced arm volume	
<i>minutes</i>	<i>cc</i>	
15	72.0	
25	72.1	
35	72.0	
45	71.9	
55-85	20 cm pressure on arm	
86	70.5	
96	71.1	
110	71.9	
120	72.0	



period of venous congestion with 15, 20, 30, 40 and 50 cm water pressure. A circle or dot represents a determination of reduced arm volume, each of which required the complete stoppage of circulation during 4 or 5 minutes. The lines therefore represent the change in the volume of tissue fluid in relation to the time when the blood was actually flowing through the arm. This period alone is of significance in relation to filtration or absorption.

During the control period the maximum variation in reduced arm volume was slightly less than 1 cc per 100 cc of arm. With a venous pressure of 15 cm water there was no demonstrable change in reduced arm volume, but with a venous pressure of 20 cm it had increased at the end of 30 minutes by over 2 cc per 100 cc. This increase in reduced arm volume was greater with higher venous pressures, amounting to almost 2 cc per 100 cc after 30 minutes' congestion with 50 cm water pressure. The details of an experiment with a venous pressure of 20 cm water are given in Protocol 1.

The fact that the higher degrees of congestion produced a greater distention of the vessels and a correspondingly greater accumulation of blood in the arm made it necessary to determine whether this factor in itself affected the reduced volume measurements. It was conceivable that reduced arm volume might be increased after congestion merely because more blood was imprisoned in the arm prior to the determination. Table 1, however, shows clearly that this was not the case. In a series of

TABLE 1

*Effect of duration of congestion on arm volume and reduced arm volume*

Subject	Venous pressure	Duration of congestion	Arm volume	Temperature of plethysmograph	Change in volume during congestion		Rate of filtration
					Arm volume	Reduced arm volume	
	cm water	minutes	cc	° C	cc	cc	cc per minute per 100 cc
A T	50	5	445	27.1	15	1.4	0.63
		10	505	27.5	28	4.7	0.93
		20	530	26.8	23	6.6	0.64
		30	520	27.6	27	11.6	0.75
E L	50	5	640	29.4	31	2.9	0.91
		10	680	29.4	31	5.4	0.79
		20	690	29.5	42	12.3	0.89
		30	650	30.5	37	12.9	0.66

4 separate experiments on each subject venous pressure was elevated to 50 cm water for 5, 10, 20 and 30 minutes. Arm volume was observed during the period of congestion, and the difference between the volume immediately before the congestion was begun and the volume at the

end of the congestion period represents the sum of (a) the volume of blood retained in the vessels and (b) the volume of the fluid which has been filtered into the tissue spaces. For comparison with this figure, the change in reduced arm volume produced by the same periods of congestion are also given in Table 1. It is evident that the change in arm volume had no constant relation to the duration of congestion, while the change in reduced arm volume clearly depended on the duration of congestion. From this comparison it is safe to state that the increase in reduced arm volume is not directly related to the volume of blood contained in the congested vessels, but is due primarily to the accumulation of tissue fluid.

The rates of filtration in a larger series of observations on both subjects have been computed and charted in Fig. 6 to show the relation between

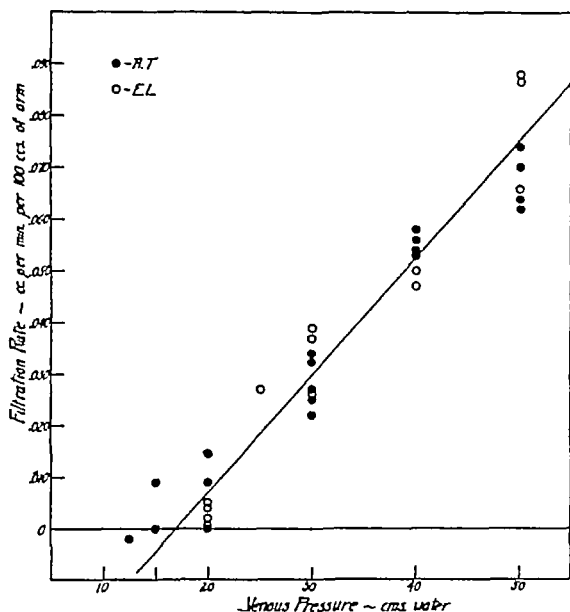


FIG. 6. CHART SHOWING RELATION BETWEEN RATE OF FLUID FILTRATION (CC PER MINUTE PER 100 CC OF ARM) AND VENOUS PRESSURE (CM WATER).

venous pressure and filtration rate. This chart includes experiments in which the congestion periods were between 15 and 30 minutes in duration.

Each subject showed in one experiment no filtration with a venous pressure of 20 cm. water, but more often filtration was observed at this

pressure Filtration was always present at venous pressures of 30 cm or over This indicates that there is a definite though small margin of safety against the production of edema in man, in that under the temperature conditions existing in these observations venous pressure could be elevated to 15 cm or even to 20 cm water before fluid accumulated in the tissue spaces

It is difficult to explain satisfactorily the variations in the rate of filtration observed at any one venous pressure Drury and Jones (1927) have shown that the rate of filtration during venous congestion increases as the temperature of the arm is raised In the experiments charted in Fig 6 the average temperatures of the arm during the filtration period varied less than 4 degrees, and most of the determinations were made within a range of 2 degrees There is some indication that the variability of filtration rate may at times be partly explained by differences in temperature, but there is no general relationship in our data between higher temperature and the higher filtration rates It is probable that another factor was more important The filtration rate per 100 cc of arm must be related to the extent of the filtering area in the arm, which must depend upon the number of open capillaries and their diameters Local factors, temperature among others, were controlled as far as possible, but central variations in vasomotor tone could not be excluded and the variations in filtration rate may well be related to differences in the filtering area produced in this way

The variations in filtration rate, though distinctly outside the limits of experimental error, were not sufficient to cause any overlap in the values observed at 20, 30, 40 and 50 cm water pressure As may be seen in Fig 6 the average point at which filtration begins is apparently 17 cm water, and above this pressure the rate of filtration is directly proportional to the difference between venous pressure and 17 cm water This straight line relationship between venous pressure and filtration rate permits the calculation of the average increase in filtration rate which is produced by an increment of 1 cm water pressure, above 17 cm water This, determined from the slope of the line in Fig 6, amounts to 0.023 cc per minute per 100 cc of arm per cm water pressure This will be compared later with a similar figure computed for the reduction in filtration rate with increase in the osmotic pressure of the plasma proteins

(c) *The removal of fluid from the tissues of the forearm* Since a venous pressure of 20 cm water causes an accumulation of fluid in the tissue spaces, the same pressure should prevent the absorption of fluid from the tissue spaces, or even produce further accumulation The term "removal of fluid" has been used in the heading of this section because it has been impossible to separate definitely the "absorption" of fluid by the capillaries from the "drainage" of fluid by the lymphatic vessels The measurements of reduced arm volume could demonstrate merely that fluid

left the arm, not the path it took in leaving. Nevertheless the effect of venous congestion on the process of restoring the normal fluid content of the tissue spaces suggests that absorption is more important with small accumulations and that lymphatic drainage is more important with larger accumulations.

The rate at which fluid was removed from the tissues is indicated in the right hand portion of Fig 5, where volume readings were continued after the congestion period, until all or nearly all of the fluid had been removed. The individual readings do not provide uniformly smooth curves because of the experimental error in single determinations of relatively small changes in volume, and because the accuracy of the determinations diminished as the subject became fatigued.

The period required for the removal of an accumulation of fluid depended, as would be expected, on the amount of fluid present in the tissues. The larger accumulations, however, were removed at a slightly more rapid rate than the smaller accumulations. This is shown in Table 2, where the average rate of removal of fluid has been calculated from 14 curves of the type shown in Fig 5. If more than 6 cc of fluid per 100 cc. of arm was present the fluid was removed at the average rate of .040 cc. per minute per 100 cc., with variations ranging from .009 to .078. If less than 6 cc of fluid per 100 cc. of arm was present the rate of removal was .026 cc. per minute per 100 cc., with variations between .012 and .047. In later observations (Fig 7) the rates observed in the two subjects were almost identical, though in earlier experiments E L showed slower rates and certain irregularities. The latter were probably due to the use of a plethysmograph which fitted the arm too closely.

Knowing the normal rate for the removal of fluid with venous pressures between 12 and 14 cm. of water (Fig 5), the effect of venous congestion on this process was tested (Fig 7). With the subject recumbent readings were taken in the usual way during a control period to demonstrate that the volume of the arm was not changing significantly. A pressure of 50 cm. water was applied to the upper arm for 5, 10, 20 or 30 minutes in order to produce small and large accumulations of fluid. The amount of fluid in the tissues at the end of the congestion period was determined. Immediately after the circulation was released at the end of this reading, the large armlet was distended by a pressure of 15 cm. water. This degree of congestion was continued for 15 minutes. One minute was allowed to elapse in order that the blood previously imprisoned in the arm might escape. At the end of this minute reduced arm volume was determined again, and followed immediately by a 15 minute period of venous congestion at 20 cm. water pressure. The procedure was repeated for a third period of 15 minutes with congestion of 30 cm. water, after which the reduced arm volume was again measured. Finally, the process of fluid removal was permitted to continue without congestion, while venous pressure was measured at frequent intervals.

The results of eight experiments of this kind are presented in Fig 7 which shows that the influence of venous congestion on the removal of fluid is also affected by the amount of the extravascular fluid present. A venous pressure of 15 cm. water conspicuously diminished, but did not entirely stop, the removal of fluid when the amount in the tissues was less than 6 cc per 100 cc of arm. Above this point 15 cm. pressure had less effect, and with the largest accumulations the rate of removal was in no way different from that observed without congestion.

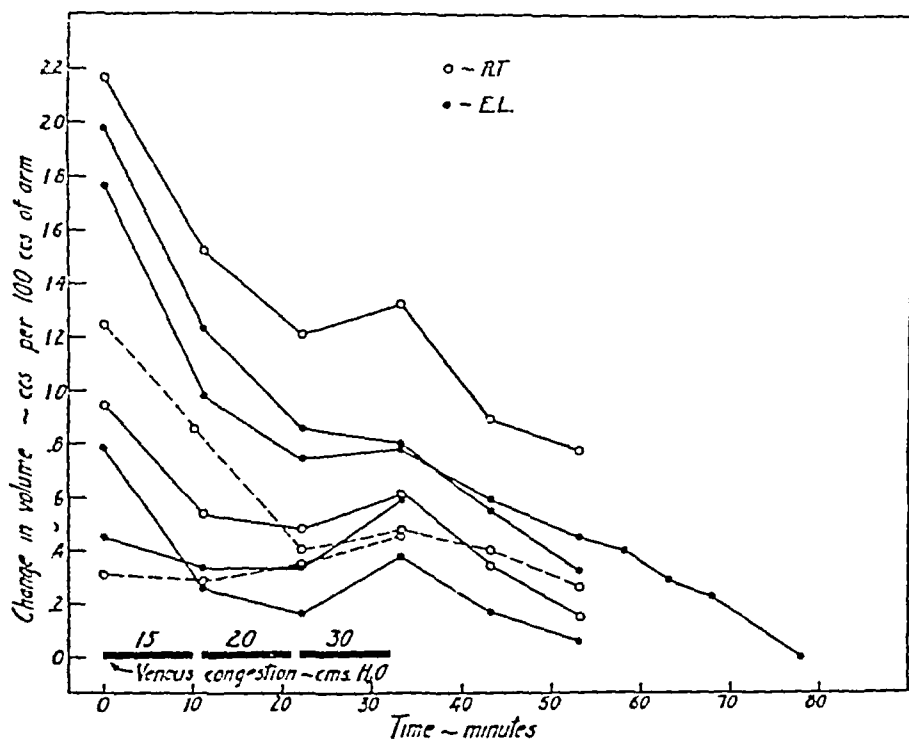


FIG 7 CHART SHOWING THE EFFECT OF VENOUS CONGESTION ON THE REMOVAL OF TISSUE FLUID FROM THE ARM

Each dot or circle represents a determination of reduced arm volume, requiring from 4 to 6 minutes' stoppage of circulation

A pressure of 20 cm. water diminished the rate of removal of smaller accumulations conspicuously and of the larger accumulations only slightly. A pressure of 30 cm. water produced filtration in all but one experiment in which a very slow removal of fluid was observed.

The average rates at which fluid was removed under these conditions have been calculated from the curves in Fig 7 and summarized in Table 2. It is obvious that the process of fluid removal was, with small accumulations, modified by 20, or even by 15 cm. water pressure, which suggests that true absorption through the capillary wall is responsible for the removal of small collections of tissue fluid.

TABLE 2

*Effect of venous congestion on removal of tissue fluid*

Venous pressure	Rate of fluid removal	
	With less than .6 cc. of fluid per 100 cc. of arm	With more than .6 cc. of fluid per 100 cc. of arm
<i>cm water</i>	<i>cc per minute per 100 cc</i>	<i>cc per minute per 100 cc</i>
12-14	026	040
15	007	055
20	002	034
30	(018)*	(003)*

( ) \*—Accumulation of fluid instead of removal of fluid

When the amount of fluid in the arm was greater, however, the effect of each congestion pressure on the rate of removal was conspicuously less. A venous pressure of 30 cm water increased the volume of tissue fluid at rates from 022 to 039 cc. per minute per 100 cc. in a forearm in which no previous filtration had occurred. The highest rate observed with this grade of congestion superimposed on an earlier filtration was 022 cc. per minute per 100 cc. The average filtration rate observed with 30 cm water pressure in the presence of a previous accumulation of fluid was about half that produced in the absence of such an accumulation. Moreover, with very large accumulations of tissue fluid, 30 cm water was observed to lose completely its power of producing an increase in the volume of tissue fluid. It appears from these observations that the presence of an accumulation of fluid in the tissue spaces reduces the power of the lesser grades of venous congestion to produce a further increase in the volume of tissue fluid. There is some mechanism which facilitates the removal of fluid and which makes further accumulation more difficult. The relation of these findings to tissue turgor and to lymph drainage will be discussed below.

(d) *The colloid osmotic pressure of the blood and the rate of fluid filtration during venous congestion.* The change from the reclining to the standing position is followed by the filtration of water and diffusible constituents from the blood into the tissues of the lower extremities (Thompson, Thompson and Dailey, 1928, Harrop and Waterfield, 1930) with a consequent increase in the blood protein concentration. The colloid osmotic pressure of the blood may be increased by this procedure by as much as 12 cm of water, as reported by Ni and Rehberg (1931), though in their experiments water intake was considerably increased.

In a series of six experiments the colloid osmotic pressure of the blood was elevated by standing, and the rates of filtration into the arm tissues were then determined for comparison with those observed in the same subjects when recumbent. It was found that with a given venous pressure the filtration in the arm of the standing subject was uniformly less

Three observations were made on each of the two subjects, with venous congestions of 25, 30 and 40 cm water. The experiments were performed in pairs so that on one day the filtration was determined both in the recumbent and in the standing position, with the single exception of Experiment 2 (Table 3). The salient features of a complete experiment are outlined in Protocol 2. The subjects stood with their backs against

# PROTOCOL 2

*February 5, 1931 Subject E L Plethysmograph on right arm*

<i>Time</i>	<i>Remarks</i>
10 00 A M	Subject began standing against board inclined at 75° Forearm at level of suprasternal notch
10 30-11 00 A M	Difficulties in adjustment of plethysmograph Readings of reduced arm volume inconstant
11 15 A M	Reduced arm volume 71.6 cc
11 26 A M	Reduced arm volume 71.7
11 36 A M	Reduced arm volume 71.4 (Average of control readings 71.6)
11 46 A M to 12 01 P M	Venous congestion of 40 cm water pressure
12 02 P M	Reduced arm volume 68.7 cc (Volume increased 2.9 cc)
12 15 P M	Blood sample (Protein 8.5 per cent, colloid osmotic pressure 43.7 cm water)
12 16 P M	Volume of arm segment 635 cc. Filtration rate 0.30 cc per minute per 100 cc of arm Observation terminated
2 25 P M	Subject reclined Plethysmograph adjusted Readings of reduced arm volume at first inconstant
2 55 P M	Reduced arm volume 84.6 cc
3 05 P M	Reduced arm volume 84.9
3 15 P M	Reduced arm volume 85.1 (Average of control readings 84.9)
3 25-3 40 P M	Venous congestion of 40 cm water pressure
3 41 P M	Reduced arm volume 80.6 cc (Volume increased 4.3 cc)
3 55 P M	Blood sample (Protein 7.6 per cent, colloid osmotic pressure 38.7 cm water)
3 56 P M	Volume of arm segment 605 cc Filtration rate 0.47 cc per minute per 100 cc of arm Observation terminated

a support inclined at an angle of 75° to the floor. The hydrostatic and vascular changes in this position are practically identical with those of the completely vertical position (Turner, Newton and Haynes, 1930), while the subjects could stand more quietly and for longer times than would otherwise have been possible. The greater muscular relaxation thus obtained was advantageous not only because it increased the accumulation of fluid in the legs (Harrop and Waterfield, 1930) but also because it permitted more accurate volume determinations. The arm was supported on a high table, with the upper surface of the forearm at the level of the suprasternal notch. The flexion of shoulder and elbow was adjusted to the position of greatest comfort.

While the subject was standing the plethysmograph was filled and adjusted as in the reclining experiments. The burette was raised on its standard so that the surface of the water could be kept level with the

TABLE 3  
The relation between the colloid osmotic pressure of the blood and the rate of filtration

Experi- ment number	Subject	Position	Time filtration began	Venous pressure		Plethya- mograph tempera- ture	Arm vol- ume	Total filtra- tion	Filtration rate	Colloid osmotic pressure of blood	Blood pro- tein	Change in		Unit change in filtration rate
				Height	Duration							Filtration rate	Colloid osmotic pressure	
				cm. H <sub>2</sub> O	min secs		cc.	cc	cc. per minute per 100 cc.	cm water	per cent	cc. per minute per 100 cc	cm H <sub>2</sub> O	cc. per minute per 100 cc
1	E L	Standing	11 40 A.M.	40	15	C.	635	2.9	0.30	43.7*	8.5*	0.17	5.0	0.034
		Reclining	3 30 P.M.	40	15		605	4.3	0.47	38.7*	7.6*			
2	A T	Reclining		40	15-30				0.56†	38.7*†	7.9*†	0.29	8.7	0.033
3	E. L	Standing	2.30 P.M.	40	15		490	2.0	0.27	47.4*	8.8*	0.15	3.3	0.045
		Reclining	5 00 P.M.	30	30	29.1	675	4.5	0.22	40.2†	8.8†			
4	A T	Reclining	10 40 A.M.	30	15	28.2	675	7.4	0.37	36.9†	8.2†	0.32		
		Reclining	3 10 P.M.	30	15	27.2	480	1.8	0.25					
5	A T	Standing	10.55 A.M.	30	15	27.8	500	-0.5	—	0.07				
		Reclining	3 30 P.M.	30	15	28.3	540	1.6	0.20	38.6†	8.5†	0.14	5.1	0.027
		Reclining	12 45 P.M.	25	30	27.8	520	2.6	0.34	33.5†	7.5†			
6	E L.	Standing	4 40 P.M.	25	30	29.3	690	0.0	0.00	43.1†	8.9†	0.27	6.9	0.039
		Reclining		25	30	27.5	690	5.6	0.27	36.2†	7.8†			

\* Blood plasma (oxalate)

† Blood serum

‡ Average of reclining experiments



upper surface of the plethysmograph. In the control period readings were taken as usual until the consecutive readings were constant. At the time when venous congestion was begun the subjects had been standing for periods ranging from 53 to 102 minutes. Venous congestion of 25, 30 or 40 cm. water was applied for 15 or 30 minutes. One minute after this congestion was released another determination of reduced arm volume was made. As soon as possible after this a blood sample was removed from an antecubital vein, without congestion except for the few seconds required to introduce the needle into the vein. Protein percentage was estimated by the Zeiss Eintauch-Refraktometer, and the colloid osmotic pressure of the blood was determined by the method of Krogh and Nakazawa (1927), using plasma with ovalate in experiments 1 and 2, and blood serum in the remainder.

The subjects, after a rest period of 1 to 2 hours, reclined and the determination of filtration rate was repeated. A second blood sample was removed after this congestion period and treated in the same way as the first sample. The order of the experiments was arranged so that the reclining observation usually followed the standing observation, which permitted double comparison with the reclining filtration rates. The two experiments were separated by a rest period several times longer than was required to absorb the fluid filtered during the first congestion, yet it was barely possible that even slight filtration several hours before might modify the rate of filtration in the second observation. When the standing observation preceded it was possible to compare standing filtration not only with the control done on the same day, but also with the maximum range of variation in the reclining position, shown in Fig. 6. In addition, however, the order was reversed in experiment 4 (Table 3) without effect on the result.

The six pairs of observations are summarized in Table 3. The filtration rates observed in the standing position were without exception lower than the lowest filtration rates observed in earlier reclining experiments (Fig. 6) on the same subjects. Comparison of the paired observations shows that colloid osmotic pressure was from 3.3 to 8.7 cm. water higher in the standing position, while the rates of filtration were from .014 to .032 cc. per minute per 100 cc. lower.

It was mentioned above that a rise of 1 cm. water in venous pressure (above 17 cm. water) increased the average filtration rate by .0023 cc. per minute per 100 cc. When the change in filtration rate in the standing position is divided by the change in colloid osmotic pressure it is found that a rise of 1 cm. water in colloid osmotic pressure is accompanied by a decrease in filtration rate of .0027 to .0045 cc. per minute per 100 cc.

The colloid osmotic pressure of the blood was also increased in the recumbent subject by venous congestion of the legs. Two large pneumatic cuffs were placed around the thighs and inflated to a pressure of

65 cm water for a period of 2 to 3 hours. Filtration rates were determined at intervals while the blood was being concentrated and compared with repeated colloid osmotic pressure determinations. There was always a diminution in the rate of filtration as the colloid osmotic pressure of the blood increased. The change in filtration per unit change in colloid osmotic pressure was in one instance below 0.023, but in all other instances above, agreeing with the standing experiments. The total change in colloid osmotic pressure was less and the results were therefore much less uniform than those obtained during standing.

#### DISCUSSION

It was realized throughout these studies that a method which required as much modification of circulation as the one described above must be controlled carefully to avoid improper conclusions based on artefacts. It has already been shown in connection with Fig. 3 and Table 1 that within the limits of these experiments variations in the amount of blood contained in the vessels prior to the determination did not introduce significant errors into the final readings of reduced arm volume. Two other possible sources of error remain to be considered: first, the possible filtration produced by the reactive hyperemia which followed each determination of reduced arm volume, and second, the possible squeezing out of tissue fluid by the external pressure applied to the arm.

The reactive hyperemia which followed each 4 to 6 minute period of occlusion elevated venous pressure in the hand (Protocol 1) only during the first 1 to 2 minutes of the recovery period. This venous pressure, always less than 27 cm water, was not sufficient to modify the arm volume permanently as shown by the constancy of the readings during the control period which preceded each experiment. The small amount of fluid filtered during the minute or two when venous pressure was thus elevated could easily be reabsorbed in the period when venous pressure was again below 15 cm water.

It was also possible to show that the application of 55 cm water pressure to the skin did not express a significant volume of fluid from the segment of arm within the plethysmograph. The lymphatic vessels were doubtless emptied in the same way as the blood vessels, and it is very unlikely that tissue fluid could be expressed by 55 cm water pressure into lymph or blood capillaries collapsed by the same pressure. The direct movement of fluid through the tissue spaces out of the arm segment was prevented by the small external bags above and below the plethysmograph which were also inflated to 55 cm water pressure. Moreover, if fluid had been mechanically expressed during the periods when external pressure was applied it would be expected that when reduced arm volumes were determined more frequently the removal of fluid should be hastened. This was tested several times and, as shown

to the right in Fig 7, the rate of removal was not appreciably raised in the period when volume determinations were made twice as frequently

The purpose of the experiments was to study certain factors in fluid balance between blood and tissue spaces under physiological conditions, and for that reason low venous pressures and the smallest possible amounts of fluid were dealt with. Filtration was observed with degrees of venous congestion which must exist normally when the arm hangs relaxed at the side of the body. The total amounts of fluid accumulating in the tissues were never more than  $1/4$  the amount required to produce a pitting edema. The term "edema fluid" has not been used, since the word edema ordinarily refers to pathological accumulations of fluid, which are large enough to distend the tissues visibly, or at least large enough to pit on pressure.

The results demonstrate that fluid accumulates in the tissue spaces when average venous pressure is above 17 cm water. This indicates the existence of a small but definite margin of safety through which venous pressure may be increased without gross disturbance of fluid balance. A similar margin of safety has been observed in certain clinical conditions which are accompanied by a reduction of the colloid osmotic pressure of the blood below its normal value of about 36 cm water. Iversen and Nakazawa (1927) state that edema appeared in ambulant patients when the colloid osmotic pressure was reduced to between 24 and 27 cm water, while Mayrs (1926) observed that after recovery from nephritis edema disappeared when the colloid osmotic pressure rose to between 20 and 23 cm water. In a case of inanition edema Landis and Leopold (1930) found that the edema disappeared when the colloid osmotic pressure was elevated to 24 cm water by blood transfusion. Thus the colloid osmotic pressure of the blood must be reduced by from 9 to 16 cm water before edema appears, while venous pressure must be elevated to between 15 and 20 cm water before fluid begins to accumulate in the tissue spaces. As might be expected the agreement is better in the comparison with recumbent patients (Mayrs, 1926, Landis and Leopold, 1930) than with ambulant patients (Iversen and Nakazawa, 1927).

This margin of safety is much less than that found by Mende (1919) in certain experiments involving venous congestion. The venous pressures which he found to be associated with the first measurable filtration must have been too high since change in limb volume was estimated by measuring the circumference of the limb, a method which is totally inadequate for the detection of small accumulations of fluid. In addition the congestion was produced by a narrow pneumatic cuff so that true venous pressure must have been significantly less than the pressure in the cuff itself.

The relationship between the gradient of capillary pressure and the colloid osmotic pressure of the blood offers an explanation of this margin

of safety According to direct measurements in human skin (Landis, 1930) average capillary pressure in the arteriolar loop is 43 cm water and in the venous loop, 16 cm water The normal colloid osmotic pressure of the blood is about 35 to 36 cm water Thus blood pressure in the arteriolar end of the capillary system is only 7 to 8 cm water higher than the colloid osmotic pressure of the blood, while capillary pressure in the venous end of the capillary system is from 19 to 20 cm lower than colloid osmotic pressure, the difference becoming still greater in the subcapillary venous plexus, or in the first venous network Even if the filtering and absorbing surfaces were exactly equal in area, the balance would favor absorption, as long as the capillary wall was almost or completely impermeable to protein It is practically certain also in the case of skin, and quite likely in the case of muscle, that the absorbing surface is greater in extent than the filtering surface This would probably provide a slight margin of safety even if the capillary wall in mammals were normally permeable to protein to the extent claimed by Harrop and Waterfield (1930) and by Drinker and Field (1931)

To produce a preponderance of filtration it would be necessary to raise venous pressure until the average difference between capillary pressure and colloid osmotic pressure in the absorbing area was slightly less than the average difference between these two pressures in the filtering area With the figures given above this should occur at slightly over 25 cm water pressure Actually, tissue fluid might be expected to accumulate at a slightly lower pressure since venous congestion must also raise the pressure in the arteriolar end of the capillary network to a slight extent

The effectiveness of 20 and 30 cm water pressure in preventing the removal of fluid from the tissues, when the total accumulation was less than 6 cc per 100 cc. of arm, makes it likely that with such small accumulations the fluid is removed chiefly by true absorption This must depend primarily upon the slight excess of colloid osmotic pressure over average capillary pressure, when the capillary area is considered as a whole It is possible to compute the average pressure through the whole capillary area from this rate of absorption A difference of 1 cm water in venous pressure changed the filtration rate by 0.023 cc per minute per 100 cc (Fig 6) Normal absorption (Table 2) occurred at the rate of 0.26 cc per minute per 100 cc Average pressure through the whole capillary network and the subcapillary venous plexus must therefore have been 11 cm water lower than average colloid osmotic pressure With a colloid osmotic pressure of 35 to 36 cm water, average capillary pressure must have been about 24 to 25 cm water This agrees with the direct measurements (Landis, 1930), in which average pressure in the arteriolar loop was 43 cm water, and in the venous loop 16 cm water A difference of 1 cm water in colloid osmotic pressure produced an average change in filtration

rate of 0.036 cc per minute per 100 cc, and this figure, used in a similar calculation, indicates that capillary pressure was between 28 and 29 cm water

When more than 6 cc of fluid per 100 cc had been filtered, venous congestions of 15, 20 and at times even of 30 cm water pressure failed to cause an increase in the volume of tissue fluid. With a venous pressure of 15 cm water tissue fluid was removed at the usual rate, and even 20 cm water pressure diminished the rate of removal very slightly. This in itself indicates that some other factor was favoring the removal of fluid and hindering a further increase in the volume of tissue fluid. This can be shown also by a calculation similar to that given in the preceding paragraph. In Table 2 it is shown that with a venous pressure of 15 cm water the fluid was removed at the average rate of 0.55 cc per minute per 100 cc. If this were done by absorption alone it would require an average capillary pressure between 11 and 12 cm water. Venous pressure, however, was 15 cm water and since capillary pressure cannot be less than this, it is obvious that another factor must be involved in the removal of these larger volumes of fluid. Similar calculations applied to the movement of fluid during congestion at 20 and 30 cm lead to the same conclusion.

Two factors that may be concerned with the more rapid removal of the larger collections of fluid are tissue turgor and lymphatic drainage. It is unlikely that tissue turgor plays an important part because it would require tissue pressures of as much as 15 cm water to modify the rate of fluid removal to the extent shown in Table 2. It is more likely, when larger amounts of fluid have collected in the tissue spaces, that fluid passes into the lymphatic vessels at a rate which can exceed the rates of filtration produced by venous pressures of 15, 20, or 30 cm of water.

The rate at which edema forms when the veins of the leg are congested has been studied by Drury and Jones (1927). They used an ordinary plethysmograph but by prolonging the periods of observation to 30 minutes, and by using relatively high grades of venous congestion (40 to 80 mm Hg), they apparently avoided the irregularities resulting from spontaneous vasomotor variations. They showed that when temperature was increased  $10^{\circ}\text{C}$  the rates of filtration became conspicuously higher. Comparison can be made therefore only with the filtration rates they observed at the temperature of  $26^{\circ}\text{C}$ . The filtration rates observed in the leg at high venous pressures were very much less than those observed in the arm at lower venous pressures. This must be related to the greater proportion of bone in the leg and particularly in the foot, which would reduce the rate of filtration per 100 cc of tissue. Only three congestion pressures were used by Drury and Jones, but the corresponding filtration rates fell on a straight line, in agreement with the findings in the arm. Moreover, from the slope of this line, it may be concluded that at  $26^{\circ}\text{C}$

a venous pressure somewhat less than 20 cm of water would be sufficient to produce the accumulation of fluid in the tissue spaces of the leg

The change in filtration rate accompanying a change of 1 cm water in venous pressure was 0.023 cc. per minute per 100 cc. The change in filtration rate accompanying the same unit change in the colloid osmotic pressure of the blood was 0.027 to 0.045 cc. per minute per 100 cc. The filtration differences are of the same order of magnitude but nevertheless a rise of 1 cm. in colloid osmotic pressure was accompanied uniformly by a greater change in filtration than was the case with a corresponding change in venous pressure. All other factors remaining constant it would be expected theoretically that the effect of unit change in these two forces would be identical. It is probable, however, that extraneous factors have made the first figure lower, and the second figure higher, than the correct value.

When venous pressure is raised by 1 cm. water, it does not follow that general capillary pressure throughout the entire network is also raised by the same amount, because of the gradient of pressure fall in the capillary network. As blood flow is diminished by the higher grades of congestion the pressure gradient in the capillary network would be reduced and the blood in the filtering area would be renewed at a slower rate. Both of these changes would tend to diminish the slope of the straight line in Fig. 6. This is compatible even with a straight line relationship between venous pressure and filtration rate since the correspondence between venous and capillary pressures must depend upon the grade of venous congestion. The real change in filtration rate which accompanies a change of 1 cm. water in capillary pressure is therefore probably slightly greater than 0.023 cc. per minute per 100 cc.

It is certain that standing modifies the mechanics of circulation and that the vascular system responds with varying efficiency to changes in posture (Turner, Newton and Haynes, 1930). In the standing position the volume of blood available for general circulation must be reduced not only by the blood distending the abdominal and leg veins, but also by the fluid which filters into the leg tissues. After prolonged periods in the standing position the diminution of circulating blood volume may produce dizziness or actual fainting. In one subject (A. T.) it was necessary to diminish the dizziness by slow movements of the legs during the latter part of the standing periods. This dizziness or fainting indicates that blood flow in the more elevated portions of the body must be reduced by the stagnation of blood in the more dependent parts. A diminution of blood flow through the arm would tend to flatten the gradient of capillary pressure in those portions of the network where it exceeded the congestion pressure. Moreover, less fluid would be filtered at a given venous pressure because the blood in the arm would be renewed at a slower rate. Both changes would tend to diminish the filtration of fluid. Since stand

ing produced not only an increase in colloid osmotic pressure, but also circulatory changes of the nature described, the change in filtration rate was probably greater than that produced by colloid osmotic pressure changes alone. An attempt to dissociate the change in filtration due to the vascular factor from that due to change in colloid osmotic pressure was unsuccessful since in the normal subject an increase in blood colloid osmotic pressure could be obtained only by methods which also diminished the volume of the circulating blood.

It seems probable that a rise of 1 cm in colloid osmotic pressure is accompanied by a diminution of filtration rate which is something less than 0.027 to 0.045 cc per minute per 100 cc, while a rise of 1 cm in capillary pressure is accompanied by an increase of filtration rate which is something more than 0.023 cc per minute per 100 cc. The true change in filtration rate produced by these two forces should be numerically identical, and probably lies somewhere between the two figures observed. The experiments present no evidence which is incompatible with the application of the Starling conception of fluid balance to man.

The above calculations could be made only with the basic assumption that the normal capillary wall is at most very slightly permeable to protein, so that the effective colloid osmotic pressure is practically equivalent to the total colloid osmotic pressure of the blood. But if tissue fluid and lymph are wholly identical as claimed by Drinker and Field (1931) the outer surface of the capillary wall must be bathed in a fluid containing between 2 and 3 per cent of protein.

It is difficult to associate the margin of safety observed in the studies described above with the very low effective colloid osmotic pressure that must result from the presence of an extravascular fluid containing from  $1/3$  to  $1/2$  as much protein as blood. In addition, if the fluid, as filtered through the capillary wall, had contained from 2 to 3 per cent protein, the absorption of the smaller accumulations of tissue fluid (Fig. 7) should have been stopped by this protein and arm volume should not have returned to normal. It would be expected, too, that with such a reduction in effective colloid osmotic pressure, the change in filtration rate per unit change of total colloid osmotic pressure should have been less than the change in filtration rate with unit change of venous pressure, whereas actually the reverse was found.

A possible mechanism of lymph formation suggested by Drinker and Field (1931, p. 36) is that "the filtrate from the blood capillaries to the tissue spaces contains water, salts and sugar in the concentrations of the blood, together with serum globulin, serum albumin and fibrinogen in low concentration, lower probably than that of tissue fluid or lymph, that water and salts are reabsorbed by the blood vessels and the protein enters the lymphatics together with water and salts in the concentration existing in the tissue fluid at the moment of lymphatic entrance."

According to this view, however, tissue fluid must vary from a filtrate with low protein content, immediately about the filtering capillary area, to a concentrated fluid containing more protein, near the absorbing areas and adjacent to the lymphatics. Lymph, being composed of the latter type of tissue fluid, must usually have a higher protein concentration than the average tissue fluid bathing the outside of the capillary wall. Analyses of lymph cannot, in general, provide definite information concerning the average protein content of the tissue fluid surrounding the capillary wall. The protein in lymph must vary widely, depending, among other things, on the rate of filtration and the relative amount of absorption. The slower the filtration and the more complete the reabsorption, the more will the composition of lymph differ from that of the capillary filtrate, and from that of average tissue fluid. The more rapid the filtration and the less complete the reabsorption, the more nearly will both average tissue fluid and lymph resemble the fluid filtered through the capillary wall.

In certain mechanical filtration edemas the extravascular fluid has been found to contain very low concentrations of protein, even considerably below 0.1 per cent (Beckmann, 1921, Achard, 1930, Landis and Leopold, 1930). In edema of this type the pressure conditions favor filtration and probably diminish absorption considerably. The composition of the tissue fluid must therefore resemble that of the capillary filtrate quite closely.

From the data available at present, it seems most likely that the normal capillary wall is not absolutely impermeable to protein, and that an exceedingly slight leakage of protein occurs rather generally. The concentration of protein in the capillary filtrate can certainly be less than 0.1 per cent, and the relatively high protein content of lymph is probably due to reabsorption of water and salts from the original capillary filtrate. The changes in filtration rate produced by changes in venous pressure and in the colloid osmotic pressure of the blood are in accord with this view.

#### SUMMARY

The movement of fluid through the human capillary wall was studied by means of a pressure plethysmograph, which collapsed the blood vessels and thus permitted the accurate determination of small changes in tissue volume. It was shown that within certain limits the determinations of volume change were not significantly influenced by hyperemia or by previous engorgement of the veins.

Fluid accumulated in the tissue spaces when venous pressure was greater than 15 or 20 cm. water. Above an average venous pressure of 17 cm. water the rate of filtration was directly proportional to the increase in venous pressure. A unit rise in venous pressure (1 cm. water) increased the filtration rate by 0.023 cc. per minute per 100 cc. of arm.



The rate at which fluid was removed from the tissue spaces depended on the size of the accumulation of fluid, being distinctly more rapid with large amounts. When less than 6 cc of fluid per 100 cc of arm was present, the removal of fluid was retarded by elevating venous pressure to 15 or 20 cm water, which was taken to indicate that small amounts of fluid were removed chiefly by true absorption. When more than 6 cc of fluid per 100 cc of arm was present the fluid was removed in spite of slight grades of venous congestion. In this connection the relative importance of tissue turgor and lymphatic drainage is briefly considered.

When the colloid osmotic pressure of the blood was elevated by standing, the rate of filtration produced by a given venous pressure was uniformly lower. A unit rise in colloid osmotic pressure (1 cm water) was accompanied by a fall in filtration rate varying between .0027 and .0045 cc per minute per 100 cc of arm.

The observations are discussed with reference to capillary pressure and fluid balance in man.

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# THE PLASMA PROTEINS IN RELATION TO BLOOD HYDRATION

## VII A NOTE ON THE PROTEINS IN ACUTE NEPHRITIS

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The preceding paper (1) of this series dealt with the serum proteins in chronic nephritis of the nephrotic type. A close relation was established between the concentration of protein in the serum and the occurrence of edema. Evidence was presented to show that the serum protein reduction which involves only the albumin fraction was chiefly the product of two factors: loss of serum albumin in the urine and malnutrition.

In this paper a similar analysis will be made of the serum proteins in acute nephritis. The total serum proteins were determined 85 times on 38 patients, in 55 instances in 22 cases the albumin and globulin fractions were separately determined. The ages of the patients ranged from 2 to 60 years. The infections which preceded and were presumably the etiological factors provoking nephritis, as well as the outcome of the disease, are tabulated below.

Antecedent infection	Number of cases				
	Total	Died	Cured	Improved	Unimproved
Scarlet fever	6	2	2	2	0
Tonsillitis and other upper respiratory infections	16*	2	7*	5	2
Rheumatic fever	4	0	3	1	0
Lobar pneumonia	5	1	1	2	1
Empyema	1	1	0	0	0
Autohemoagglutination	1	1	0	0	0
Unknown	5	1	3	0	1
	38				

\* One of these patients also had severe impetigo contagiosa.

The great majority of patients who have been labelled "improved" probably recovered completely, but could not be followed long enough to make this certain. Of the "unimproved" there is reason to believe that

most or all died or developed the chronic form of the disease. The second group, "tonsillitis or upper respiratory infections," is chiefly composed of patients with hemolytic streptococcus infections. Some of them probably belong in the scarlet fever category, but escaped because they presented no exanthematous lesions. The disease in one of the patients in the scarlet fever group began after a sore throat which was unaccompanied by any rash. Because her sister had scarlet fever, the hemolytic streptococci recovered from the throat of the patient were tested and found to be of the scarlet fever variety.

The disturbances of the serum proteins appear to be related neither to the ages of the patients nor to the etiological agents concerned in the production of the disease.

Figure 1 shows the level of the serum proteins, protein fractions and the oncotic pressure in all observations, and the relation of each one of these factors to the occurrence of edema. As in the chronic nephrotic forms of the disease edema was always present when the total protein concentration was less than 4 per cent and, with one exception, when albumin was below 2.20 per cent. On the other hand, it often occurred when the protein and albumin concentrations were above 5 per cent, and occasionally even when they were well within the normal limits. The natural inference to be drawn from the figure is that while oncotic pressure is quite as influential in the production of edema in acute nephritis as it is in the chronic nephrotic condition, some additional factor is active in the acute condition. Moore and Van Slyke (2) and others (3, 4) have reported similar observations.

In Figure 2 the same phenomenon has been illustrated in a different manner. In this figure the abscissae represent the length of time which has elapsed since the onset of nephritic symptoms, the ordinates the serum protein concentration. Solid circles indicate edema. Near the onset of the disease edema is found often enough when the serum proteins are high. As the disease progresses, however, edema disappears unless the proteins fall. It is, of course, impossible to draw sweeping conclusions from such semi-statistical treatment of the data. The majority of the patients who maintained a high protein level throughout the disease recovered rapidly. Therefore, towards the right of the figure, the hollow circles at high levels represent chiefly convalescent or recovered cases, the solid circles at low levels cases that failed to improve or passed through a long nephrotic stage. The development of serum protein deficiency in the latter may be only evidence of advance of the disease, the disappearance of edema in the former, evidence of recovery. The records of certain individual cases in the series make this explanation unsatisfactory.

Number 68285, male, aged 48, was admitted on the 17th day of the disease with a profuse albuminuria, blood pressure of 150/110 and a blood nonprotein nitrogen of 39 mgm per 100 cc. He had moderate subcutane-

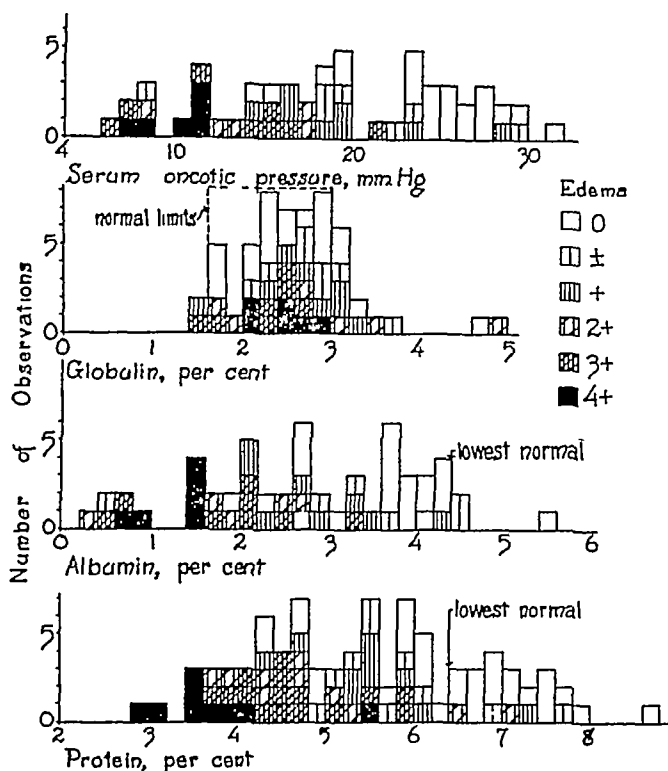


FIG 1 THE RELATION OF SERUM PROTEINS TO EDEMA IN ACUTE NEPHRITIS

Estimation of the degree of edema is quite rough  $\pm$  indicates slight puffiness of the eyes, or slight swelling of the feet noticed by ambulatory patients at the end of the day + represents persistent, demonstrable swelling of the feet 2+, more extensive subcutaneous edema 3+, subcutaneous edema and serous effusions 4+ extreme general anasarca

Oncotic pressure was calculated from the serum protein values by the factors of Govaerts (6) ( $5.5 \times$  per cent albumin) + (1.4 per cent globulin) = oncotic pressure in mm Hg

ous edema, but diuresis had already actively begun. The serum proteins were 6.18 per cent, albumin 3.84 per cent. The edema showed no tendency to recur although the serum protein as late as the 46th day was only 5.92 per cent, with only 3.69 per cent of albumin. Meanwhile the blood pressure had fallen to normal, but the proteinuria continued undiminished. In this case edema appeared and disappeared in the course of the disease without relation to the serum proteins, which never fell far below the normal limits.

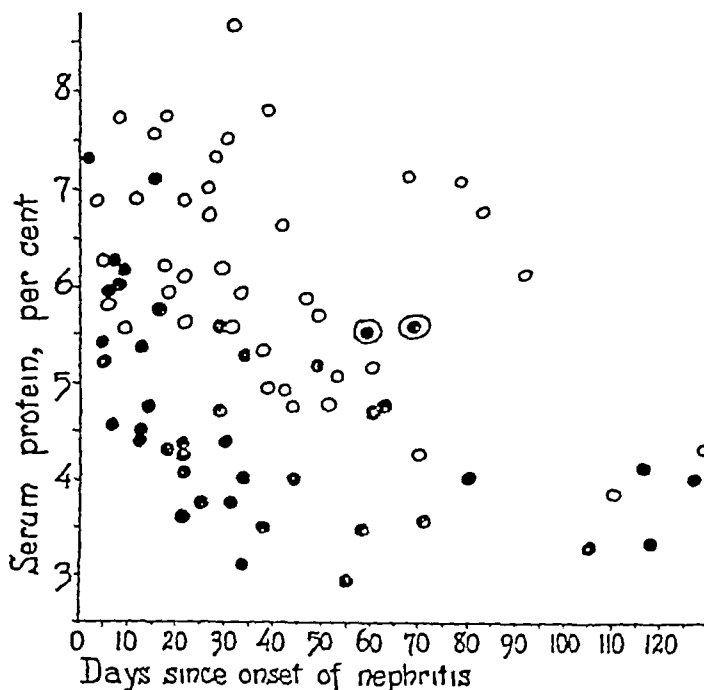


FIG. 2 THE RELATION OF SERUM PROTEINS AND EDEMA TO THE DURATION OF THE DISEASE

Solid dots represent patients with edema, open circles—patients without edema, ringed dots—patients with distinct evidence of heart failure with edema.

In several cases proteins at the first examination were found somewhat reduced and rose later in the course of the illness, while proteinuria and other evidences of nephritis continued. In these instances edema disappeared as it does in nephrosis cases when the protein in its upward course approached 5 per cent.

If Starling's theory of edema production is accepted, the appearance of edema with a normal serum oncotic pressure must be due either to increased vascular permeability, which permits proteins as well as a protein-free filtrate to pass through the capillary walls, or to increased capillary blood pressure. There can be little doubt, in view of the retinal lesions, the hypertension and other circulatory changes, that the patho-

logical manifestations of acute nephritis are not confined to the kidney, but involve the general vasculature of the body. It is probably to these vascular disturbances that the edema is to be referred. Whether increased vascular permeability or increased hydrostatic pressure is chiefly responsible for the exudation it is still impossible to say. Available analyses of serous effusions from acute nephritic patients reveal only slightly higher concentrations of proteins—about 0.6–0.7 per cent—than are usually found in similar effusions from nephrosis patients ((5, 7) and authors' unpublished data). These are not so high as those found in effusions in heart failure. Unfortunately these determinations have not been made at the inception of the disease.

In this connection attention may be called to the two solid circles which have been distinguished by rings. These two are exceptions to the general rule that edema persists in late stages of the disease only when the proteins are greatly reduced. Both patients had distinct evidences of heart failure, in one of them associated with hemolytic streptococcus septicemia.

The causes of albumin reduction, when this occurs, seem to be the same as those which are operative in the chronic nephrotic forms of nephritis: drainage of serum albumin into the urine and malnutrition, in the sense of protein starvation. Anorexia, nausea, vomiting and digestive disturbances are among the commonest symptoms and are assisted in their destructive work by the infection which so commonly accompanies at least the early stages of the disease. In this series when the patients were able to take diets containing adequate amounts of protein and high calories serum albumin failed to fall or rose if it was low. It fell only when digestive disturbances or the restrictive efforts of physicians limited diets. The value of such restrictions is quite doubtful unless it be assumed that malnutrition and consequent edema are desirable aims of therapy. At least limitation of protein and calories should be practised only over short periods in selected cases.

A further point, to which attention has been called by previous observers, is brought out in Figure 1, the relative frequency of high globulin values. These are presumably referable, not to the renal lesion, but to the infectious process from which it originates and with which it is associated. There is no demonstrable relation between the concentration of globulin and the occurrence of edema.

#### CONCLUSIONS

Serum proteins have been determined 85 times in 38 cases of acute nephritis.

Edema was regularly found when the protein concentration was below 4 per cent, but was sometimes present in the early stages of the disease when protein and albumin were within or just below the normal limits.



In the early stages of the disease edema appears to be due partly to vascular disturbances which increase the hydrostatic pressure or the permeability of the capillaries

Serum albumin reductions, when they occur, seem to be referable to leakage of albumin into the urine and malnutrition

Serum globulin in acute nephritis is frequently above normal

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# THE PLASMA PROTEINS IN RELATION TO BLOOD HYDRATION

## VIII SERUM PROTEINS IN HEART DISEASE

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In uncomplicated heart disease the proteins of the serum appear to be little disturbed. In patients with heart failure, on the other hand, they may be considerably reduced at the expense of the albumin fraction (1, 2, 3). Attempts to relate these reductions to the incidence of edema or the state of the water balance have not been successful. The protein concentration may fall during diuresis or rise during the development of edema. In some cases, however, changes in the opposite direction have been observed.

In the hope that more extensive studies might throw some light on the nature of these apparently paradoxical phenomena, 53 determinations of the serum proteins were made on 24 patients with heart disease in various stages of decompensation. In 49 instances albumin and globulin fractions were determined separately. Efforts were made to estimate the nutritional state of each subject. In 2 cases nitrogen metabolism was determined while the subjects were recovering from cardiac decompensation. The nature of the cases included in the series is tabulated briefly below. In none was renal function seriously impaired. The concentrations of

TABLE 1  
*Classification of patients*

Nature of disease	Number of cases			
	Total	Improved	Unimproved	Died
Arteriosclerotic without hypertension	8	5	1	2
Arteriosclerotic with hypertension	6	5	0	1
Rheumatic	6	2	1	3
Syphilitic	3	1	0	2
Acute endocarditis	1	1	0	0

<sup>1</sup> The data in this paper are taken from the thesis of Dr. Sheldon A. Payne, done in partial fulfillment of the requirement for the degree of Doctor of Medicine at Yale University School of Medicine.

protein and protein fractions of the serum bore no relation to the fundamental nature of the heart disease, but seemed to be related only to functional disturbances connected with heart disease in general

All the data are presented in Figure 1, which is designed to show the relation of both decompensation and edema to the concentration of total

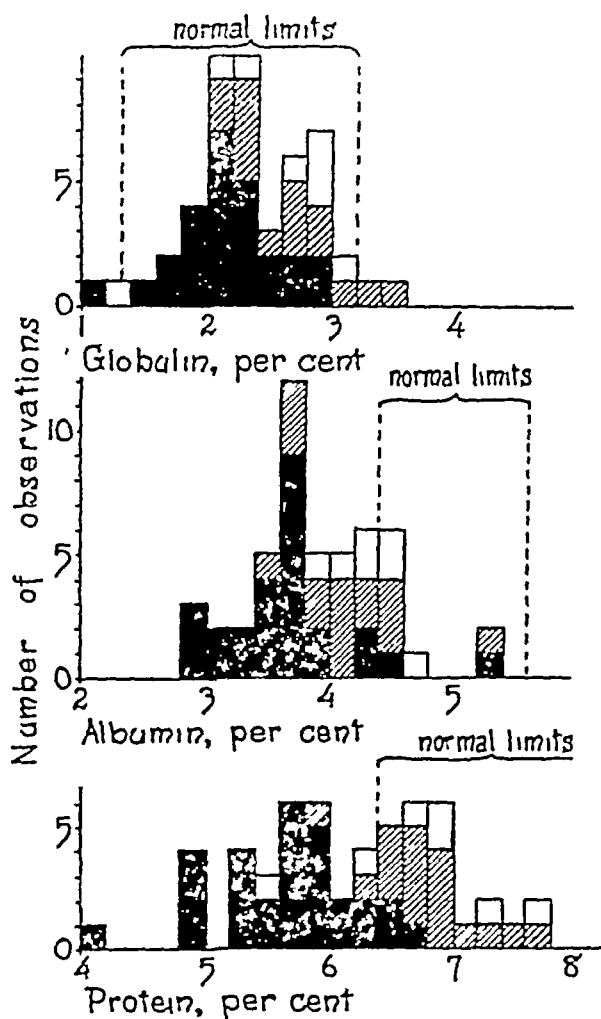


FIG 1 RELATION OF SERUM PROTEINS TO EDEMA AND TO HEART FAILURE

Solid squares indicate heart failure with edema diagonally lined squares, heart failure without edema, open squares, no heart failure nor edema

protein and of the protein fractions of the serum The presence of decompensation and edema is indicated by the solid squares, decompensation without edema by diagonal lines The criteria by which decompensation has been distinguished in these cases are quite arbitrary Patients have been considered as compensated only when they were sufficiently well to be discharged from the hospital

If all observations are considered, total proteins and albumin are found to be reduced in the majority of instances, while globulin does not depart appreciably from the normal range. The proteins in compensated cases were, with one exception, within, or not more than 0.2 per cent below, the limits of normal variation. This exception had only 5.55 per cent protein. However, albumin accounted for 4.24 per cent of this, the protein reduction being due merely to an unusually low globulin, only 1.31 per cent. Albumin also lay within or near the normal limits when compensation had been established.

The incidence of edema becomes greater as protein and albumin fall. However, it can not be inferred that the two are related, because it has already been shown that the serum proteins return to the normal level with the establishment of compensation, whether there has been edema or not. There is, however, other evidence that edema is more frequent in patients with hypoalbuminemia. Seven of the patients were admitted with all signs of heart failure except edema. In this group total protein lay always above the lower normal limit, albumin in only two instances below 4.00 per cent, 3.61 and 3.83.

Reduction of the serum proteins may be referable to any one or all of a variety of functional disturbances which are known to occur in heart disease. The ones which appear most worthy of consideration are albuminuria, hydremia, filtration of protein through the capillary walls into the edema fluid, and malnutrition. Of these albuminuria seems to play a relatively unimportant rôle. None of the cases studied had profuse albuminuria, and in only 5 did the urine contain more than a faint trace of albumin.

If hydremia were responsible for the protein reductions, both protein fractions and not albumin alone, should suffer. That alterations of blood volume are encountered in heart failure and that such alterations do affect the blood proteins is apparent in some of the cases which will be discussed in greater detail below. They are not, however, a frequent cause of hypoproteinemia.

Analyses of serous effusions from patients with heart failure have revealed the fact that such fluids contain more protein than comparable effusions from patients with nephrosis (4, 5). This suggests that capillary permeability is somewhat increased in heart failure, presumably as a result of blood stagnation and anoxemia. If, from the same analyses, the total amounts of protein which may pass from the blood stream into the edema fluids are estimated, the quantities seem hardly sufficient to strain the regenerative powers of a normal individual. Iversen and Johansen (5) found in the early stages of heart failure about 0.6 per cent of protein in pleural fluid. Subsequently the protein may rise considerably, as fluid is reabsorbed more rapidly than protein is removed. If 0.6 per cent represents the average concentration of protein in cardiac edema fluid,

the development of as much as 10 kilos of edema would remove from the serum only 60 grams of protein. An equal amount may be excreted in the urine of a nephrotic patient in the course of only 3 or 4 days. Removal of comparable quantities from dogs by plasmapheresis produces only transient serum protein deficiency (6, 7)

TABLE 2  
*Serum data, edema and weight*

Case number	Serum			Edema	Weight			Remarks
	Protein	Albumin	Globulin		Actual	Before illness	With out edema	
	per cent	per cent	per cent		kilos	kilos	kilos	
80788	7.72	5.35	2.37	0	53.6			Patient noted as slightly obese
48764	7.40	4.38	3.02	0	43.7	43-48		
6099	7.30	3.88	3.42	0	64.8	73-77		
80853	7.01	5.30	1.71	+	88.0	84-86	85.1	
73410	6.92	4.08	2.87	0	82.4	82		Weight reported normal by patient Vomiting and dehydrated
60084	6.91	4.05	2.86	0	41.6			
4607	6.85	3.61	3.24	0	50.2	59-64		Had lost 8 kilos during preceding year. Study made after improvement
87471	6.65	4.44	2.21	0	47.0	55		
73840	6.50	3.62	2.88	+	58.8	73	52.2	
85828	6.45	3.93	2.52	3+	76.8	73	63.8	
82039	6.40	3.73	2.67	0	64.0	68		
79555	6.16	3.46	2.70	2+	70.2	70	60.4	
80060	6.05	3.72	2.33	3+	66.9	73	54.0	
85900	5.83	3.63	2.20	+	69.5	70-77	66.8	
76591	5.83	2.89	2.94	2+				Just before admission patient had noted 22 kilos loss of weight. He was evidently emaciated.
87704	5.76	4.59	1.17	+	68.2		63.9	
73795	5.75	3.75	2.00	4+	86.3+	75	71.3	Patient a woman of medium height, well nourished even after elimination of edema
82756	5.63	3.55	2.08	2+	55.4	59-64		
81512	5.51	3.16	1.90	2+				
24341	5.34	2.96	2.38	3+	59.9	64-68	52.6	Emaciated and unable to take food or fluids
56404	5.30			3+				
54174	4.97			3+	62.8	69	50.4	Emaciation marked
82465	4.97	3.42	1.55	2+				
63611	4.96	2.92	2.04	2+	57.4	64	54.0	

\* Patient could not be weighed until two days after this determination

Table 2 gives the protein values in each case at the time of the first observation when there was obvious heart failure. The presence and degree of edema are also noted. Finally an attempt has been made to estimate the nutritional state of each patient. These estimations are

based on comparisons between the weights of the patients before they became ill, while they were suffering from heart failure with edema, and, in those that improved, after edema had disappeared. The data are arranged according to the serum protein values. General inspection of the weight records and remarks shows quite conclusively that the first patients, those with high proteins, are normally or well nourished, while those with low proteins are distinctly malnourished. Either their weights with edema are little in excess of the normal or else the weights after diuresis give evidence of considerable emaciation.

Certain exceptions to the rule deserve especial consideration. Number 60084, with a weight of 41.6 kilos, was obviously emaciated. His serum proteins, however, were quite normal, 6.91 per cent, and albumin was 4.05 per cent. The patient was desperately ill and vomiting so continuously that he was unable to take food or fluids and had become dehydrated. The high protein and albumin figures are probably the result of hemoconcentration. The relatively high globulin value, 2.86 per cent, supports this explanation. Numbers 6099 and 4607 have high proteins in spite of weight losses of 10 or more kilos. In these cases, however, albumin is distinctly reduced, 3.88 and 3.61 per cent, the protein concentration being maintained by abnormally high globulin, 3.42 and 3.24 per cent. Number 87471 again had normal proteins, although he was 8 kilos below normal weight. Strictly speaking this case should be omitted from the table because the determination was not made until two weeks after hospital admission, during which there had been steady clinical improvement and return of compensation. Number 87704 represents an exception of the opposite nature. In this case the proteins were low without evidence of malnutrition. Albumin, however, is quite normal, the protein deficiency is entirely referable to reduction of globulin.

The effect of malnutrition on serum proteins appears to be confined entirely to the albumin fraction (8). For this reason better correlation is found, in accordance with expectation, between the state of nutrition and the concentration of serum albumin. In the 11 observations with albumin below 4 per cent, when normal weight could be compared with edema free weight, there was found to have been a weight loss of from 3 to 21 kilos. With the two exceptions noted above, 60084 and 87471, patients with albumin greater than 4 per cent had suffered no loss of weight.

Such consistent correlation seems sufficient proof that the serum albumin deficits of heart failure are due to malnutrition. The histories leave no doubt that anorexia is the chief cause of the malnutrition, with nausea and vomiting frequently acting as contributory factors.

The nature of the protein fluctuations in heart failure is further elucidated by certain individual cases in which repeated observations were made.

Number 82039 (see Figure 2), a male, 60 years old, with syphilitic heart disease and aortic insufficiency, was admitted to the hospital January 4, 1930, without edema, but suffering from severe dyspnea and orthopnea which had developed progressively during the preceding four weeks. His weight, which had previously been 68 kilos, was only 64. Serum albumin was only 3.73 per cent, although total protein was within normal limits. He responded well to treatment and was discharged from the hospital, without symptoms or signs of heart failure, January 29, 1930. At home his condition rapidly deteriorated again. February 18, he was readmitted with the previous symptoms and, in addition, extensive subcutaneous edema. In spite of the edema he weighed only 67.5 kilos.

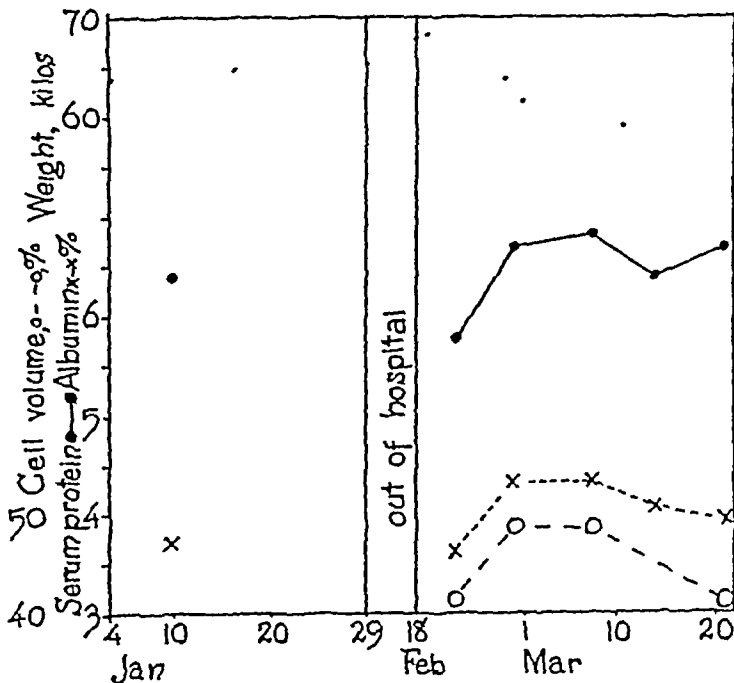


FIG 2 CASE NUMBER 82039 COURSE OF SERUM PROTEINS DURING RECOVERY FROM HEART FAILURE

Albumin had fallen to 3.63 per cent. Under treatment with rest, digitalis and restricted fluids edema diminished rapidly. By February 28 there was only slight pitting of the ankles, and by March 7 all edema had disappeared. The weight, meanwhile, had fallen, first to 62, finally to 60.5 kilos. Although there is little indication in these weights that nutrition had improved, albumin had risen to 6.40 per cent. At first sight this would seem to refute the theory that the albumin deficiency is connected with the state of nutrition. In this case blood cell volumes were determined by means of the hematocrit. If the cell volumes are compared with the protein values, it will be seen that albumin and cell volume rise together during the period of digitalis diuresis and fluid restriction, to

fall again when edema has been eliminated and more liberal amounts of fluid are given. The natural deduction is that the albumin increase is due to temporary hemoconcentration. This inference appears the more probable, because globulin and albumin rise together.

Similar evidences of temporary hemoconcentration are seen in other cases, especially when fluids are restricted during periods of diuresis. The kidneys in these cases appear to excrete fluid from the serum more rapidly than it is withdrawn from the tissues. During this period of blood concentration, in the case under discussion, blood nonprotein nitrogen rose from 23 to 50 mgm per 100 cc in spite of clinical improvement, to fall subsequently, even when the dietary protein was increased. This phenomenon of dehydration azotemia is well recognized.

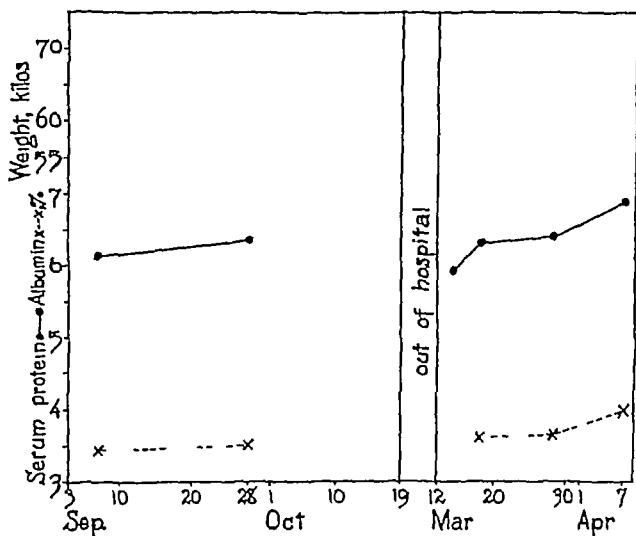


FIG 3 CASE NUMBER 79555 COURSE OF SERUM PROTEINS DURING RECOVERY FROM HEART FAILURE

Number 79555 (see Figure 3), a male, 80 years old, with arteriosclerotic heart disease and slight hypertension, was admitted to the hospital September 3, 1929, with severe dyspnea, orthopnea, cyanosis, enlargement of the liver, pulmonary congestion and massive edema of the lower extremities and the lower part of the trunk. On September 7, when his condition was little altered, serum protein was 6.16 per cent, with albumin 3.46. With all his edema he weighed at this time no more than he had weighed before his illness. Under treatment he improved rapidly, and was dis-



charged on October 19, free from edema. September 28, at the end of his diuresis, his weight had fallen to 60.5 kilos, 10 kilos below his normal weight. The serum albumin had not changed appreciably. March 14, he was readmitted with all his previous symptoms and signs and, in addition, ascites. He was too ill to be weighed. The serum protein had fallen still further. In view of his record of weight loss he was given, from the first, a diet containing 80 grams of protein and 2500 calories. With this and other therapeutic measures he had a rapid and profuse diuresis. During the diuresis the proteins rose somewhat in spite of the extreme loss of weight.

After diuresis was apparently complete albumin rose rapidly from 3.68 to 4.02 per cent, although the weight did not increase. Determinations of the nitrogen metabolism during the period between the last two observations show that the patient was daily storing nitrogen equivalent to a little more than 30 grams of protein. Restoration of the body protein stores was, therefore, proceeding rapidly. In recovery from famine edema Ling's (9) data show that reconstitution of serum albumin occurs rapidly after administration of adequate diets and may be complete before body weight has returned to the normal level. The same course of events, illustrated by this case, and, to a lesser extent by the preceding one, is seen during the recovery period in other patients who were subjected to frequent examinations.

#### DISCUSSION

According to Starling's (10) theory passage of fluid between the blood stream and the tissue results from the interplay of two forces: the hydrostatic force of the capillary blood pressure, which tends to drive fluid out of the vessels, and the osmotic pressure of the non-diffusible colloids, chiefly serum proteins, which tends to draw fluid back from the tissue spaces into the blood stream. In nephrosis and in malnutrition reduction of the oncotic pressure is chiefly responsible for the production of edema, which has been found to occur only when the serum proteins, especially serum albumin, fall below a certain level (8). In heart failure, presumably, increase of capillary blood pressure, the result of venous congestion, is the main cause of edema. For this reason exact correlation between serum oncotic pressure and edema production is neither to be expected nor found.

Edema may be associated with any protein or albumin concentration. One can conceive of extreme conditions in which hydrostatic pressure became so great that fluid was forced from the vessels until proteins rose far above the normal level and a high grade of hemoconcentration developed in the presence of edema. In a moderate degree such a state is illustrated by the vomiting case, 60084, noted above, and during diuresis and fluid restriction, in 82039 (see Figure 2 and text above). A more

striking instance was observed in a patient with arteriosclerosis involving both heart and kidneys. The subject, a woman of 60, when admitted to the hospital, had general anasarca and was vomiting frequently. The serum protein concentration at this time was 9.13 per cent, the red blood cell volume 33.6 per cent, the oxygen capacity of the blood 17.3 volumes per cent. As she improved under rest and digitalis the proteins fell to 7.90 per cent, the cell volume to 28.9 per cent, and the oxygen capacity to 15.1 volumes per cent. The parallel fall in all 3 functions is almost conclusive evidence that the serum protein changes during this period were due chiefly or only to dilution of the blood to its normal volume by fluids withdrawn from the edematous tissues.

Although edema may, if capillary blood pressure rises significantly, occur at any protein value, its production can proceed only to the point where the mean capillary pressure equals the oncotic pressure. If the latter is reduced by reason of serum protein deficiency the same degree of congestion will produce a greater degree of edema. It is not, therefore, entirely without significance that edema (Figure 1) is more consistently found in patients with low serum proteins. From a therapeutic point of view it would seem reasonable, certainly, to direct some attention to the prevention or correction of serum albumin deficits. As these appear to be referable to malnutrition, the common practice of restricting diet, and especially protein, in heart failure, may represent misdirected effort. By proper choice of foods a diet containing reasonably generous quantities of protein and adequate calories may be given in a form which will not overtax the digestion of even an elderly arteriosclerotic with heart failure. This is well illustrated in case 79555, cited above. Such a diet can also be provided without an excess of salt or fluid. Heart failure is, and should rationally be treated as, a wasting condition, with only the necessary compromises for the digestive disturbances by which it may be accompanied.

How large a part increased capillary permeability may play in the production of cardiac edema it is hard to say. Experiments of Krogh (11), Landis (12) and others have demonstrated that if venous obstruction, with consequent impairment of tissue oxygenation, becomes sufficiently great, the capillary walls will permit protein to escape. Serous effusions from patients with heart failure contain distinctly higher concentrations of protein than do the almost protein free effusions of nephrosis (4, 5). Although loss of serum proteins in this manner is probably a minor cause of serum protein depletion, it may, nevertheless, contribute distinctly to edema formation by reducing the effective oncotic pressure of the serum. This is, of course, measured, not by the actual concentration of protein in the serum, but by the difference between the concentration of protein in the serum and that in the interstitial fluids. Under normal conditions the latter is so small as to be negligible. If it becomes increased the serum protein must rise by an equal increment to balance the same capillary blood pressure.

## CONCLUSIONS

- 1 In patients with heart failure serum albumin is frequently reduced
- 2 Although edema of heart failure may occur even when serum protein and serum albumin are at or above the normal level, it is more commonly associated with some degree of albumin deficiency
- 3 The albumin deficits appear to be directly referable to malnutrition

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## THE PLASMA PROTEINS IN RELATION TO BLOOD HYDRATION

### IX SERUM PROTEINS IN THE TERMINAL STAGES OF RENAL DISEASE

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The three preceding papers of this series (1, 2, 3) have dealt with the concentration of serum proteins in chronic edematous nephritis, acute nephritis and heart failure, respectively. It has been shown that in all these conditions serum albumin deficiency is common and is related to edema production. However, the correlation between edema and albumin deficiency is exact only, in chronic edematous nephritis, in which circulatory and vascular disturbances are minimal. In all three diseases, hypoalbuminemia, irrespective of its relation to edema, appears to be due chiefly to malnutrition,—or, more exactly, protein deprivation,—which may be referable partly to proteinuria, but is usually chiefly the result of inadequate protein intake.

The present paper deals with the serum proteins in the terminal stages of renal disease. Total proteins have been determined 266 times on 61 cases, protein fractions 81 times on 24 cases. In 130 instances in 42 cases blood cell volume was determined by means of a Daland hematocrit, and oxygen capacity by the method of Van Slyke and Stadie (4) or that of Van Slyke and Hiller (5). On 35 additional occasions, in 13 cases, either oxygen capacity or cell volume was determined. In 1 case the volume of the circulating plasma was determined by the vital red method of Keith, Rowntree and Geraghty (6).

The cases can be divided roughly into 3 groups: 1—chronic glomerular nephritis, 2—arteriosclerotic nephritis, and 3—patients with destructive lesions of the kidney. The last group, which will, for convenience, be designated as “suppurative nephritis,” includes patients with bilateral pyonephrosis or pyelonephritis, bilateral renal tuberculosis, and polycystic kidneys. The nature of the underlying disease has no demonstrable effect on the serum proteins, which appear to be influenced only by the functional disturbances to which the disease gives rise. Most of the patients were studied over relatively short periods in the last stages of renal disease. A few were observed at earlier stages, some for periods

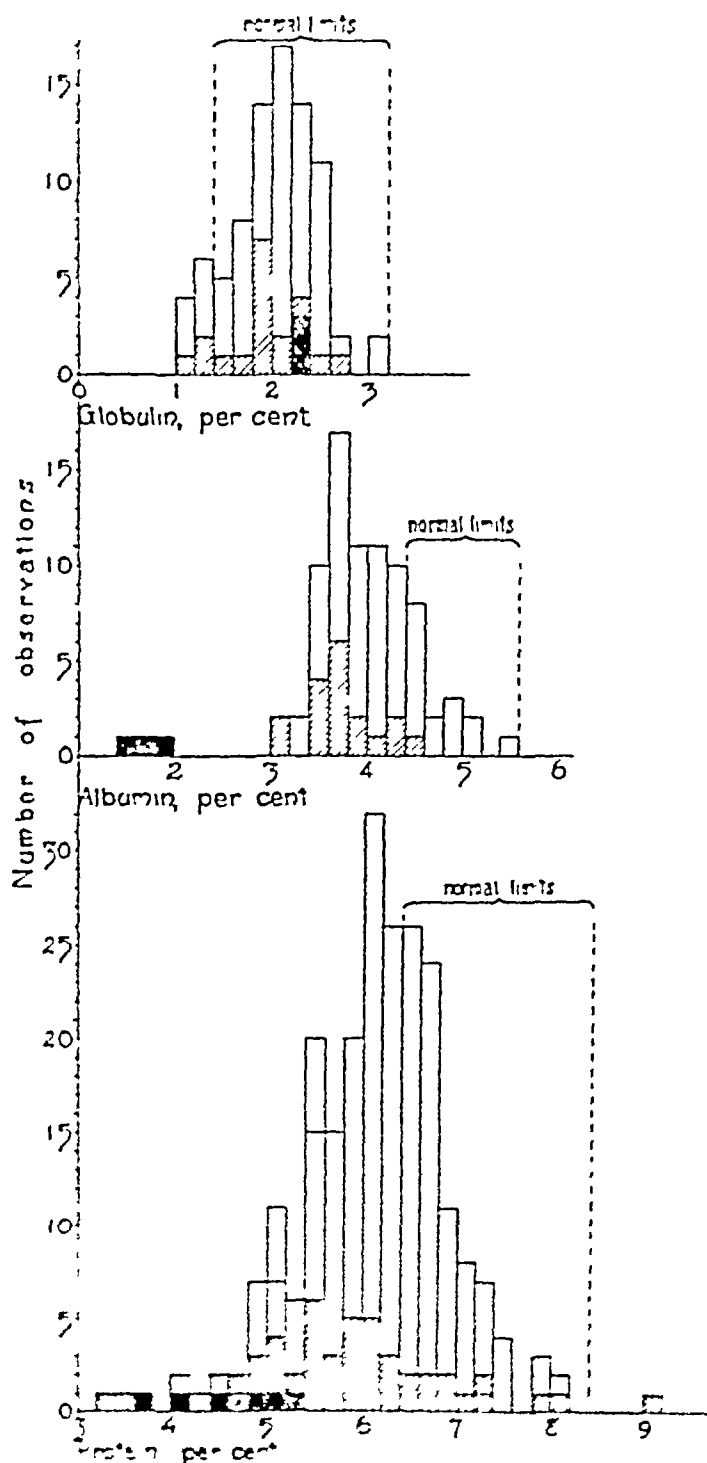


FIG. 2. THE RELATION OF SERUM PROTEINS TO EDWARDS' TERMINAL NEPHRITIS

Solid bars represent patients with heart failure diagonally lined bars represent patients with heart failure. The squares with crosses are from a patient whose terminal nephritis is due to lumbar poisoning.

of as much as 2 or 3 years. All exhibited evidence of impaired kidney function in inability to concentrate urine, most had extreme reduction of phenolsulfonephthalein excretion and high blood nonprotein nitrogen.

In Figure 1 all the protein data are presented, as they have been in the preceding papers, with especial reference to the incidence of edema. An attempt has been made, however, to distinguish between edema associated with heart failure and edema occurring without evidence of circulatory decompensation. In cases of this type such a distinction is fraught with difficulties and must, in the last analysis, rest upon the judgment of the clinical observer. In the great majority of instances, in this series, in which edema is connected with heart failure, there were unequivocal objective signs of cardiac disease with decompensation. Pericarditis, coronary occlusion, paroxysmal tachycardia, cardiac irregularities, evidence of advanced chronic passive congestion of the lungs and other organs, were the commonest of these signs, especially in the premortal stages of the disease. Dyspnea with cyanosis, but without reduction of the alkaline reserve or obvious pulmonary pathology, was considered a sufficient indication of heart failure, as was response to digitalis by clinical improvement and diuresis.

In far more than half the observations serum protein was distinctly below the normal level, with occasional deficits quite as great as those encountered in nephrosis. As in the other conditions which have been studied it is entirely the albumin fraction of the serum which suffers, globulin remaining relatively unaltered. These observations agree with those of previous observers (7, 8, 9).

At first sight there appears to be no semblance of correlation between the protein level and the incidence of edema. In fact the single patient with proteins distinctly above the normal range had general anasarca. On closer examination it becomes evident that edema is both relatively and absolutely more frequent when the proteins are reduced. What is more significant is the fact that edema without signs of heart failure is found only when there is a definite serum protein deficiency.

In the production of the hypoalbuminemia, proteinuria may play a part, at times not negligible. However, in some of the cases with greatly reduced serum albumin proteinuria has been quite insignificant. Abnormally large serum volume due to hemodilution can be excluded because the globulin is not reduced. Moreover, direct determinations of serum volume by the dye method made by Brown and Rowntree (10) revealed no tendency to hemodilution in cases of this type. As will be shown subsequently hemoconcentration is probably a far commoner occurrence.

In Figure 2 an attempt has been made to compare the serum protein concentration with the state of nutrition of the patients. The criteria which have been employed in the evaluation of the nutritive state have been discussed at length in the preceding papers (6, 7, 8). They are,

obvious emaciation, evidence of recent large weight losses and, in a few instances, records of subsistence over long periods on inadequate diets. In addition, it has been necessary to give especial consideration to vomiting, which is, in this condition, so common and so serious a symptom. In the figure, vomiting cases are distinguished from those with malnutrition due to other causes. Occasional vomiting has not been recognized, but only vomiting of such frequency, severity and duration as to prevent the oral administration of reasonable amounts of food and fluids, and to produce dehydration.

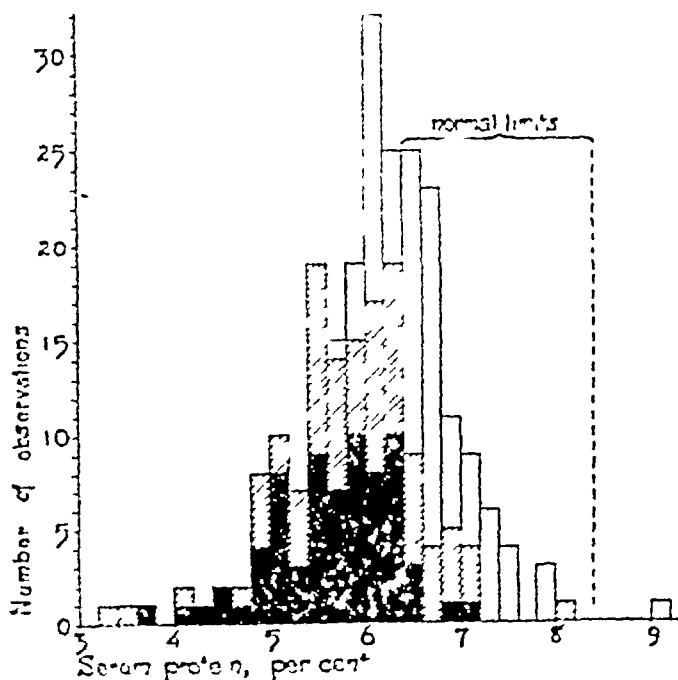


FIG. 2 THE RELATION OF SERUM PROTEIN TO MALNUTRITION IN TERMINAL NEPHRITIS

Solid squares represent malnutrition diagonally lined squares, persistent vomiting

Even if the vomiting cases be omitted there is a surprisingly large incidence of severe malnutrition with little or no protein deficiency, which can be explained only by consideration of the serum proteins in individual cases. The hemoconcentration which may result from heart failure, especially when fluids are restricted, has been discussed in the preceding paper. In that discussion one of the cases in this series, the one with proteins above the normal range in Figure 1, was mentioned. An opportunity to observe the phenomenon more exactly and frequently was presented by a group of patients with nephritis who developed acute heart failure as a terminal condition. Under these circumstances certain changes in the constitution of the blood occurred with great regularity. The serum proteins rose sharply, while blood cell volume and oxygen capacity remained unaltered or diminished. Examples of this association of events are shown in the first two cases in Table 1. In 7 of the 11 cases in which studies were made before and after sudden heart failure the serum proteins rose, the increases varying from 0.31 to 1.52 per cent. In one case they remained unchanged. In the three cases in which they fell large quantities of fluid had been given subcutaneously or intravenously shortly before the observations were made. Oxygen capacity fell in 7 out of 8 instances, in the other remaining unchanged. Cell volume fell in 7 instances, rose once and remained unchanged once. The simplest explanation for the protein increase is passage of fluid without proteins from the blood to the tissue spaces. The simultaneous reductions of cell volume and hemoglobin can hardly be due to blood cell destruction, but are probably a result of circulatory stagnation, which causes cells to become segregated in certain parts of the circulation. This concentration of the serum in heart failure accounts for a certain number of the observations in which the proteins are high in malnourished patients who were not vomiting.

Attention has been called, in another connection (11, 12), to the impairment, in advanced nephritis, of the mechanisms by which the salt and water content of the body is usually stabilized. If the salt intake of the patient with severe renal damage is restricted, dehydration results because urinary salt excretion does not diminish to the same extent that it does in normals. Restriction of fluids also leads to dehydration because of the tendency to diuresis, the polyuria of renal insufficiency. With dehydration from these causes hemoconcentration usually occurs. Alterations of serum proteins associated with variations in salt and water intake are illustrated in the third and fourth cases in Table 1.

Although there is a tendency, in individual cases, especially over short periods, for hemoglobin, cell volume and serum proteins to vary together, there is no demonstrable general correlation between serum proteins and either cell volume or hemoglobin. Profound anemia may be found in advanced nephritis without serious protein deficiency, and



## Remarks

	Weight kg.	Height cm.	Temp. °C.	Pulse	Press. mm. Hg.	Acid	Alb.	Alb. per cent	Alb. per cent	Alb. per cent	Remarks
1914											
December 14	105	61.8	7.07	23.2	12.0	0					Malnourished and dehydrated from continuous vomiting
December 19	105	60.1	6.75	23.5	9.8	0					Still eating little, but taking fluids better
December 31	178		7.06	21.2	9.5	0					Oronary occlusion on December 26 Death January 1
1919											
January 25	211		5.82	12.0	11	+					Extreme emaciation, dehydration and vomiting
January 26	253		3.41	11.1	11	2+					After large amounts of fluid subcutaneously
January 27	251		3.30	10.6	11	2+					Parenteral fluids continued
January 28	251		5.60	10.1	11	2+					Pericarditis and acute heart failure appeared June 27 Death January 29
1919											
November 20	12	51.8	6.89	12.9	17.8	0					Undernourished and vomiting steadily
November 24	39	53.1	7.23	39.1	17.3	0					After salt poor, high fluid treatment
December 5	41	52.8	6.31	36.7	16.1	0					After limited fluids with 7 grams NaCl daily
December 17	47	50.0	6.63	38.5	16.9	0					After salt poor, high fluid treatment
December 27	81		5.71	35.5	16.2	0					2 days before death Extremely emaciated
1924											
February 19	85	67.9	6.66	29.1	11.8	0					No heart failure nor vomiting
February 25	75	66.1	7.09	32.6	15.0	0					After a salt poor diet
February 29	69	66.1	6.71	32.5	11.1	0					After restricted fluids with 5 grams of NaCl daily
March 8	11	68.2	6.27	31.3	13.8	0					After high fluids with 5 grams of NaCl daily
1925											
January 11	75		6.93		12.7	0					Seen in dispensary
November 19	167		6.61	23.0	8.7	0					Readmitted, emaciated, dehydrated and vomiting
November 27	168		6.31	21.3	8.1	0					Eating food and fluids better
December 1	167		5.09	17.1	7.3	0					Vomiting Receiving large amounts of fluid and salt subcutaneously
December 6	163		6.17	17.1	5.9	0					Frequent convulsions Less fluids given
December 11	158		5.62	15.3	1.9	0					Improving Receiving large amounts of fluids
December 17	145		5.89	20.0	7.1	0					Eating and taking fluids by mouth
1926											
January 2	165		6.19	18.3	7.1	0					Eating well Taking less fluid
January 9	171		1.19	11.9	5.1	0					Pericarditis and heart failure Parenteral fluids Death January 11

low albumin with little reduction of hemoglobin. Furthermore, peculiar divergences of hemoglobin and proteins are seen in individual cases. An example of such divergence in acute heart failure has already been mentioned. Others have been found which can not be explained so easily. In the second case of Table 1, number 29039, between the first and second observations, within 24 hours, proteins fell 2.4 per cent while hemoglobin and cell volume remained practically unchanged. One can argue with no certainty that the administration of large amounts of fluids, increasing the blood volume, swept cells from stagnant portions of the circulation. In this and the preceding case determinations were made on arterial blood. This is only one instance in which, even during very short periods, the concentrations of the cellular constituents of the blood and of the protein exhibit large and entirely unrelated changes. Numerous other similar occurrences can be found in the Table.

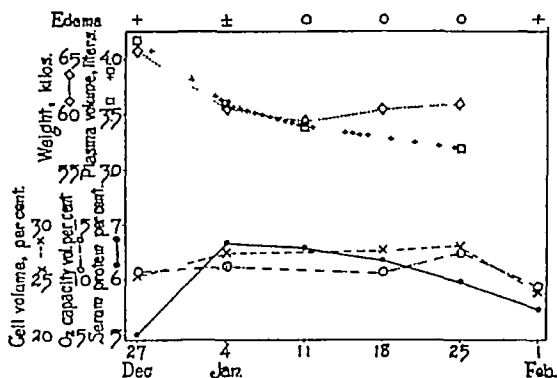


FIG 3 CHANGES OF SERUM PROTEIN, IN THE TERMINAL STAGE OF NEPHRITIS, COMPARED WITH CHANGES OF SERUM VOLUME AND BLOOD CELL VOLUME CASE NUMBER 56247

Figure 3 shows perhaps the most extraordinary example of the lack of relation between proteins and cellular constituents. In this case determinations of serum volume by the vital red method afford a more accurate basis for the analysis of the fluctuations in proteins and cells. The patient, an Italian male, aged 39, was admitted to the hospital December 26, 1926, with advanced chronic nephritis. He appeared well nourished, but had vomited continuously for some days. There was slight edema of the ankles which was ascribed to heart failure and disappeared rapidly with rest and digitalis. Vomiting ceased almost entirely after he entered the hospital, but he was not able to take large amounts of fluid and voided relatively large quantities of urine. There is an immediate rapid drop of weight with a striking reduction of plasma volume and

a simultaneous increase of serum proteins, evidently due to hemoconcentration. This reduction of plasma volume is accompanied, however, by a relatively insignificant rise of hemoglobin and cell volume. At the third observation weight and plasma volume have continued to fall, at a diminished rate. This time the proteins have also decreased. Meanwhile the nitrogen balance has been continuously negative, with losses amounting to about 16 grams of protein daily. From this time until death the serum proteins diminish steadily, the negative nitrogen balance continuing with only a brief interruption. Plasma volume, on the one occasion when it was again determined, had fallen still further, in spite of the administration of large amounts of salt and water<sup>1</sup>. The temporary rise of hemoglobin and cell volume at the next to last observation may be partly referable to a transfusion given on January 19. The gain of weight towards the latter part of the illness probably represents retention of fluid preceding the appearance of demonstrable edema, associated with heart failure. The final fall of hemoglobin and cell volume may be connected with terminal exacerbation of the heart failure. After the fifth observation, January 25, vomiting became so extreme that the patient took no food and received fluids almost entirely by hypodermoclysis or infusion. One is tempted to ascribe the initial rapid rise of serum proteins to hemoconcentration, the subsequent gradual fall to continued malnutrition. Whatever may have been the causes of the changes it is obvious that the reduction of plasma volume tends to mask the true extent of the serum protein depletion. If the total amount of circulating protein is estimated by multiplying, in each instance, the plasma volume by the serum protein concentration, the following values are obtained: December 27, 210 grams, January 4, 240 grams, January 11, 230 grams, January 25, 190 grams.

Not only do the concentrations of serum proteins and the cellular constituents of the blood often run divergent courses, even the relation of cell volume to oxygen capacity shows rapid fluctuations. It has been recognized that changes in the reaction of the blood cause the red blood cells to swell or contract. The causes of these reactions have been elucidated by Hamburger (13), Van Slyke (14) and others. In a disease in which disturbances of acid-base equilibrium are so frequent and profound, alterations of the size of the red blood cells are to be expected. They should, however, be of relatively small magnitude, while in some of the nephritic patients of this series the cell volume changes were extraordinarily large. In Table 1 can be found some examples which can not consistently be connected with variations of  $\text{CO}_2$ , which was simultaneously determined in each case.

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<sup>1</sup> This case has been discussed in another article from this department (12). In spite of the administration subcutaneously of enormous amounts of salt, in the latter part of his course, he exhibited a continuously negative chloride balance. A large part of the chloride was excreted through the bowel.

Although considerable attention has been given to the effect of blood reaction on cell size, the effect of total base concentration has not been considered. If, however, the cell membranes are, as is generally believed, impermeable to bases *in vivo* as they are *in vitro* (15), changes in the base concentration of the plasma, unless immediately mirrored by proportional changes in the base content of the cells, must of necessity be compensated by an exchange of fluid between the two phases. Cell size must change. In nephritis serum base concentration is disturbed almost, if not quite, as frequently as are serum pH and CO<sub>2</sub> (9, 10).

#### DISCUSSION

Reduction of serum protein at the expense of the albumin fraction, then, occurs with great frequency in the terminal stages of nephritis, and may become quite as great as that found in nephrosis. It can not be correlated with the incidence of edema because the latter, when it occurs in this condition, is usually referable to heart failure. Malnutrition is a characteristic feature of the disease and can be demonstrated in all cases that present hypoalbuminemia. On the other hand, serum albumin is often normal or high when there is evident malnutrition. Reasons for this are found in the tendency of these patients to develop hemoconcentration. Among the causes of hemoconcentration are heart failure, vomiting, anorexia and the inability to conserve the salt and water stores of the body. Extreme and rapid fluctuations of serum protein concentration, blood oxygen capacity and blood cell volume are produced by the interplay of these same functional disturbances, which vary the serum volume, blood cell volume and blood cell distribution. Such fluctuations often mask the true extent of the serum albumin depletion.

#### CONCLUSIONS

- 1 Reduction of serum proteins at the expense of the albumin fraction is common in the terminal stages of renal disease.
- 2 The serum albumin deficiency can not be correlated with the incidence of edema, which is usually referable to heart failure.
- 3 Wasting is a characteristic feature of the condition and can be correlated with the serum protein deficiency, unless some other functional disturbance has produced hemoconcentration.

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# THE VARIATIONS IN SERUM CALCIUM, PROTEIN, AND INORGANIC PHOSPHORUS IN EARLY AND LATE PREGNANCY, DURING PARTURITION AND THE PUERPERIUM AND IN NON-PREGNANT WOMEN

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Marriott and Howland (1) in 1916 showed that in the blood of uremic patients the serum calcium is low but that there is an increased concentration of inorganic phosphorus. That there might be some relation between the rise of phosphate and the fall of calcium was first suggested by Binger (2) who injected solutions of O phosphates intravenously into dogs and observed an elevation of inorganic phosphorus in the blood accompanied by a reduction of serum calcium, an observation later confirmed by Tidall (3). Salvesen and Linder (4) noted a parallelism between changes of protein and calcium in sera and transudates from patients with nephritis and heart disease. Among uremic patients with low serum calcium and protein, the observers also noted an enormous retention of phosphates, the decrease in calcium being proportional to the increase in phosphorus.

Peters and Erierson (5) working mainly on patients with cardiac and renal disease, evaluated statistically their observations and those of Salvesen and Linder (4) and developed a mathematical equation to express the relationship between serum calcium, inorganic phosphorus, and protein, as follows:

$$\text{Ca} \approx 7 - 0.255 \text{ P} + 0.566 \text{ protein}$$

They concluded that the concentration of calcium in the serum varies directly with the protein content and inversely with the concentration of inorganic phosphorus, and expressed the belief that in order to interpret serum calcium variations correctly, simultaneous determinations of protein and inorganic phosphorus should be made.

Sterns and Knowlton (6) failed to demonstrate a constant relationship between the level of calcium and protein, or inorganic phosphorus, in the sera of children, from birth to 16 years of age. In their study, no data were included from nephritic or cardiac patients, nor from those with obvious disturbances of calcium metabolism.

The present investigation was designed to extend the study of this relationship to early and late pregnancy, parturition, and the puerperium, as well as to normal non-pregnant women

#### METHODS AND MATERIAL

The sera from 98 individuals were examined. Those with renal or cardiac disease were excluded. The blood was collected without (or with minimum) stasis, three to four hours after the previous meal, and immediately centrifuged. The serum was removed promptly from the cells. No anti-coagulants were employed.

TABLE I

*Serum calcium and inorganic phosphorus according to total serum protein concentrations*

Non-pregnant women			Women in early pregnancy (1 to 3 months)			Women in late pregnancy (8 to 9 months)		
Total protein	Inorganic phosphorus	Calcium	Total protein	Inorganic phosphorus	Calcium	Total protein	Inorganic phosphorus	Calcium
<i>per cent</i>	<i>mgm per cent</i>	<i>mgm. per cent</i>	<i>per cent</i>	<i>mgm per cent</i>	<i>mgm per cent</i>	<i>per cent</i>	<i>mgm per cent</i>	<i>mgm per cent</i>
6.30	4.0	9.4	6.47	4.2	10.4	4.85	3.2	9.0
6.40	3.9	10.0	6.48	4.0	10.6	5.63	3.9	9.2
6.63	4.4	10.9	6.63	4.6	10.5	5.63	3.2	9.4
6.85	3.9	9.7	6.73	4.0	10.9	5.66	3.9	8.7
6.98	3.9	10.6	6.74	3.9	10.4	5.79	3.4	9.4
7.00	3.8	9.9	6.76	3.5	10.4	5.87	3.2	8.8
7.10	3.9	10.1	6.85	4.7	10.0	5.95	3.0	9.0
7.12	3.7	11.0	7.46	4.2	10.1	6.00	3.0	9.2
7.12	4.2	10.3	7.55	4.1	9.8	6.18	4.4	9.7
7.15	4.5	10.1	7.56	4.2	10.7	6.20	4.1	9.0
7.48	3.7	10.1				6.21	3.3	9.3
7.55	4.5	10.7	6.92	4.1	10.4	6.27	4.0	9.2
7.95	4.6	11.3				6.27	4.0	10.8
7.95	3.9	10.5				6.43	4.1	9.9
7.98	4.6	10.9				6.47	3.9	9.1
8.00	3.2	11.2				6.50	4.2	10.6
						6.50	3.8	9.3
7.22	4.0	10.4				6.58	4.4	10.1
						6.62	4.2	10.0
						6.63	4.1	9.5
						6.65	3.8	9.1
						6.67	3.9	9.3
						6.75	3.7	9.6
						6.75	4.1	8.9
						6.78	4.8	10.4
						6.78	2.8	9.2
						6.90	3.4	9.4
						6.94	3.8	9.9
						6.96	3.6	9.5
						7.02	3.4	9.5
						7.07	3.1	9.2
						7.10	4.9	9.0
						7.65	3.6	10.2
						6.43	3.8	9.5

TABLE I—Continued

Blood serum determinations			Urine determinations (24 hr. collection)		
Total protein	Inorganic phosphorus	Calcium	Total protein	Inorganic phosphorus	Calcium
6.10	2.8	9.7	6.6	3.7	9.5
6.20	3.1	9.9	6.77	4.0	9.0
6.21	3.2	9.4	6.84	4.9	9.6
6.00	3.2	9.5	6.96	5.2	9.3
6.07	3.3	10.8	6.97	5.0	9.5
6.07	4.2	11.0	7.01	5.0	9.6
6.10	4.3	9.5	7.04	5.3	9.5
6.16	3.6	9.9	7.11	5.1	9.5
6.20	4.2	9.9	7.24	5.1	10.3
6.24	4.8	10.1	7.2	4.5	10.5
6.26	4.5	9.5	7.17	4.5	11.0
6.33	4.4	10.2	7.55	4.3	10.8
6.38	4.5	10.3	7.65	4.8	9.8
6.40	3.5	9.9	7.69	4.6	10.3
6.45	3.8	10.4			
6.50	3.3	10.0	7.16	4.6	9.9
6.51	4.4	10.6			
6.61	4.5	9.0			
6.69	4.9	10.1			
6.76	3.7	10.4			
7.1	4.2	9.2			
7.20	4.7	9.5			
7.33	3.7	9.2			
7.51	4.5	10.9			
7.61	3.6	10.1			
6.41	4.9	9.9			

The total nitrogen was determined by the micro Kjeldahl method (7) and the determined nonprotein nitrogen subtracted to give the protein nitrogen which was then multiplied by the factor 6.25 to obtain the total protein. The inorganic phosphorus was determined by the method of Benedict and Theris (8), and the calcium by the Clark Collip (9) modification of the Kramer Tisdall (10, 11) procedure.

The sera from the five series of women studied were grouped according to the protein content (Table I). Variations in calcium and phosphorus were then compared with the changes in protein concentration.

TABLE II  
Summary

Blood serum determinations	Normal non-pregnant	Early post-nancy	Late post-nancy	Parturition	Post partum 7-9 days
Number of observations	16	10	33	25	14
Protein (average per cent)	7.2	6.9	6.4	6.4	7.2
Phosphorus (mgm. per 100 cc.)	4.0	4.1	3.8	3.9	4.6
Calcium (mgm. per 100 cc.)	10.4	10.4	9.5	9.9	9.9



## DISCUSSION

The correlation coefficients between serum protein and calcium and between serum calcium and phosphorus have been calculated from the experimental data in Table I, and are presented in Table III

TABLE III  
*Correlation coefficients*

	Protein	Calcium	Calcium	Phosphorus
Non-pregnant women	+ 0.65	$\pm 0.098$	+ 0.148	$\pm 0.167$
Early pregnancy	- 0.16	$\pm 0.152$	- 0.317	$\pm 0.211$
Late pregnancy	+ 0.355	$\pm 0.102$	+ 0.379	$\pm 0.101$
Parturient women	+ 0.082	$\pm 0.134$	+ 0.121	$\pm 0.134$
Puerperal women	+ 0.728	$\pm 0.085$	- 0.273	$\pm 0.166$

It is evident from Table III that there is a significant correlation between the protein and the calcium concentrations in the sera of non-pregnant and puerperal women only. In early pregnancy, the protein values tend to be somewhat diminished, while the serum calcium is unaffected, and the correlation completely disappears. (It is realized that the number of determinations in this group is too small to permit an accurate statistical survey.) In late pregnancy and during the post-partum period, all three constituents are reduced somewhat below the non-pregnant level, and again there is no mathematical correlation of significance.

Comparative study of the serum phosphorus and calcium values in the various groups fails to elicit any significant correlation.

A mathematical formula can be evolved expressing a relationship between any two blood constituents which normally exhibit comparatively narrow ranges. The proof of such a relationship, however, lies in the maintenance of a significant correlation between the two constituents under conditions allowing wide variation of either component. In normal non-pregnant women, the range of serum protein is slight, whereas in late pregnancy and during parturition the protein concentration tends to be lower and the range is considerably increased. The fact that in these latter groups there is no significant correlation between calcium and protein bears out the contention that the level of serum protein in these conditions is not the chief factor in determining the level of the serum calcium. The same reasoning can be applied to the relationship assumed to exist between serum calcium and inorganic phosphorus.

## SUMMARY

1 Variations of serum calcium with changes of inorganic phosphorus at different protein concentrations were studied in non-pregnant women, in early pregnancy, in late pregnancy, during parturition, and after delivery.

2 The data indicate a significant correlation between the serum protein and the serum calcium in non pregnant and puerperal women, which is completely lost in late pregnancy and parturition when the protein range is considerably widened

3 No significant correlation between the serum calcium and the serum inorganic phosphorus was observed in any of the five groups studied

#### ADDENDUM

Since the above was written, there has appeared a theoretical exposition by Greenwald (12), in which he derives a new equation for expressing the relationship between serum protein and serum calcium ( $\text{Ca} = x + 0.875 \text{ protein}$ ) Considering only the groups of normal individuals cited by Greenwald, it is found that the protein is the more variable factor, and that the calculated value for  $x$  is dependent largely upon the levels of serum protein in the group studied

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# THE EFFECT OF LIVER AND COMMERCIAL LIVER EXTRACT ON THE BODY WEIGHT, RED BLOOD CELLS, AND RETICULOCYTES OF NORMAL RATS

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The remarkable changes occurring in patients suffering from Addisonian pernicious anemia who have received adequate liver therapy are now well known. Among these changes the rise of the percentage of reticulocytes in the peripheral blood is most striking, and attention has also been repeatedly drawn to the gain in weight associated with improvement in the blood picture (1) (2).

In pigeons treated with liver preparations of known potency in pernicious anemia and given intravenously, there was an increase of immature red blood cells in the peripheral blood (3). On the other hand, normal pigeons or pigeons starved until a nutritional anemia had been produced when fed an exclusive diet of broiled liver, showed an initial gain of body weight. The normal pigeon, however, on the liver diet became gradually anemic, while the pigeons with the nutritional anemia produced by starvation showed a partial regeneration of the peripheral blood at first, then a fall of both red blood cells and hemoglobin, resulting in a severe anemia. This occurred in spite of rapid gain of weight. An exclusive meat diet fed under similar conditions seemed to be much more adequate for blood formation in pigeons (4). This has been verified by Barlow (5) with regard to meat and only partially for liver because he experienced difficulty in getting pigeons to eat the liver. In our experience the birds will eat broiled liver unless subjected to too long starvation for that particular animal.

The effects on the blood obtained in pigeons with liver fed as an exclusive diet and liver preparations fed or injected intravenously, have been interpreted as being dependent upon the megaloblastic bone marrow found in birds, a type of bone marrow found in pernicious anemia and other anemias of man amenable to liver therapy. To test this hypothesis a series of experiments have been made on rats, an animal that has been used extensively for nutritional studies, and is therefore well standardized. The rat also has a hyperplastic, active bone marrow, but it is predominantly erythroblastic and normoblastic, i.e., similar in type to

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the normal bone marrow of man The experiment was devised to give a possible answer to the following question

1 Are the changes observed in pernicious anemia and in grain fed pigeons when liver or liver preparations effective in pernicious anemia are administered, dependent upon the megaloblastic character of the bone marrow?

#### EXPERIMENTAL METHODS

Twelve healthy male rats of the same age and approximately the same weight were divided into four groups Daily weights were recorded and reticulocyte counts made every other day, using Cunningham's (6) modification of Hawes' (7) method Red cell counts were made once a week in a Neubauer chamber, using standard pipettes Hemoglobin determinations were made at the same time using Haldane's method During a short control period they were fed on a synthetic standard rat diet<sup>2</sup> supplemented by the salt mixture recommended by Osborne and Mendel (8) Group I was then given raw minced liver which was intimately mixed with the standard food so as to represent 25 per cent of the total amount fed This amount of liver was given for 15 days and then increased to 50 per cent of the total intake for a further period of 15 days Finally whole liver without any other food was given for a similar period Food was always supplied in excess

Group II was given the standard diet only throughout the whole period of the experiment, thus serving as a control group

Group III was fed daily for 15 days, 0.5 gram of liver extract number 343 (N N R) derived from 14 grams of whole liver in addition to the ordinary standard food This was later increased to one gram of liver extract number 343 (N N R), daily for 15 days

Group IV was given the waste product obtained in the preparation of a fraction potent for pernicious anemia which may have contained extremely small quantities of the effective principle

#### EXPERIMENTAL OBSERVATIONS

1 *Red blood cells* No appreciable alteration was found in the red blood cell counts of any of the animals throughout the period of investigation Variations were within normal limits, the highest figure being 9,897,000 red blood cells per c mm, and the lowest 7,720,000 In no case was there a variation greater than 1,000,000 in any one animal The same was true of the hemoglobin determinations

2 *Reticulocyte counts* There was no significant alteration in the reticulocyte count in any of the animals The greatest variation was in one rat of the control group which fluctuated between 0.3 and 6.9 per cent, but the latter count was exceptional The average figure for the whole series was 1.54 per cent

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<sup>2</sup> Casein, 20 per cent, pure Arrowroot starch, 56 per cent, fresh creamery butter, 15 per cent, dried yeast, 5 per cent, and salt mixture, 4 per cent

3 *Weight gain* The average gain in weight of the three rats receiving the standard diet which contained 25 per cent of whole liver for 15 days was 15.6 grams, compared with 13.3 grams in the control group. When the liver was increased to 50 per cent of the diet the gain in weight was 12 grams, as compared with 8.3 grams in a similar period in the control animals. The total gain in each group during the 30-day period was an average of 28.66 grams for each rat fed whole liver, and 21.66 grams in the control group. When fed whole liver alone for an 18-day period there was a loss of weight averaging 12 grams.

In Group III fed liver extract, number 343 (N N R), there was an average gain in weight of 15.2 grams in the first 15 days when a dose of 0.5 gram daily was given, and of 7.83 grams in the second period of 15 days when 1.0 gram was fed in addition to the standard diet, giving a total average gain of 22.66 grams in 30 days.

Group IV fed the waste product of an effective liver preparation made an average gain of only 5.33 grams in 15 days. This substance was extremely distasteful to the animals, and while taking it they never appeared in good condition. In a 3 week period after cessation of treatment two rats made an average gain of 28 grams, while the third, which had some lung infection as the result of inhaling some of the liver preparation while an attempt was being made to forcibly feed it, made no gain.

It would appear from the above observations that the addition of either whole liver or liver extract to the diet of healthy adult rats for a short period has no effect on either the weight (except perhaps in the rats fed whole liver), the reticulocytes, or the total number of red blood cells. It is recognized that the treatment was maintained for a short period only, but results are obtained both in patients with pernicious anemia and in pigeons during a similar short period, and the results are therefore comparable.

The gain in weight in the animals receiving liver was not apparently significantly greater than in the control group, especially when dealing with such a small series of animals.

#### DISCUSSION AND CONCLUSIONS

From the above experiments it is seen that when healthy adult rats were fed a synthetic diet supplemented with raw liver or liver extract number 343 (N N R) no change was noted in red blood cells, hemoglobin or reticulocytes. No increase in weight, considered to be significant, was observed in liver fed rats as contrasted with the controls.

It is of interest to note that Vedder (9) and others did not obtain any effect on the blood picture of rats with anemia caused by *Bartonella muris rattis* when large doses of liver extract were administered. Rats with posthemorrhagic anemia with either whole liver or liver extract added to their diet did not regenerate red blood cells and hemoglobin.

faster than the controls (10) Adlersberg and Gottsegen (11) produced a temporary anemia in healthy dogs and rabbits on an ordinary diet by feeding large doses of commercial liver preparations effective in pernicious anemia (hepatrat and hepatropson) On the other hand, Whitehead and Barlow (12) report a rapid recovery in rats suffering from rice disease when meat or liver was substituted for the rice The curative effect was better with lean beef than liver Red blood cells and hemoglobin were completely recovered with lean beef or liver two or three weeks after the body weight had been regained

It is evident that feeding of liver or commercial liver extract to healthy adult rats on an artificial standard diet, produced none of the effects on the peripheral blood and the weight, as has been observed in pernicious anemia of man and in grain fed pigeons This is suggestive, and tends to support the hypothesis that a megaloblastic bone marrow is essential to produce the effects obtained by the administration of liver or liver preparations effective in pernicious anemia

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# STUDIES IN CONGESTIVE HEART FAILURE

## XV REFLEX VERSUS CHEMICAL FACTORS IN THE PRODUCTION OF RAPID BREATHING<sup>1</sup>

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### INTRODUCTION

Dyspnea in patients with cardiac disease may assume a number of different forms. The most important of these are

A. Dyspnea upon exertion

B. Dyspnea at rest

1. Orthopnea,
2. Paroxysmal dyspnea (cardiac asthma),
3. Cheyne Stokes respiration,
4. Continuous dyspnea

The last four types of respiratory distress are likely to occur in various combinations with one another. Thus, a patient dying of congestive heart failure may feel severely short of breath at all times, even in the sitting position, but his dyspnea becomes worse when he attempts to assume the recumbent posture, and even when he sleeps sitting up, he awakens with violent paroxysmal exacerbations of the symptom. In such a patient, Cheyne Stokes respiration is often present to further confuse an already extremely complex situation. Cheyne-Stokes respiration may occur without the simultaneous presence of the other forms of dyspnea. Orthopnea may also occur in "pure" form. Cardiac asthma is, however, invariably associated with orthopnea and often with Cheyne Stokes respiration. Continuous resting dyspnea is almost always associated with one or more of the other forms of respiratory distress.

Do the various clinical forms of cardiac dyspnea have the same pathogenesis? It has been customary to say that they are all due to deficient cardiac output—a statement which is far from being proved by existing evidence. The fact that these several forms of dyspnea have distinctive clinical characteristics suggests that they are due to different physiological alterations, but this is also unproved.

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It has seemed to us that any effort to reach an understanding of cardiac dyspnea should include

- (a) a method of measuring respiratory distress,
- (b) a study of each of the various types of dyspnea in "pure" form, and
- (c) an investigation into the various combinations observed clinically

A method of measuring respiratory distress has been developed and is reported in the tenth paper of this series (Harrison, Turley, Jones and Calhoun (1931))

The dyspnea of mild exertion and that of orthopnea have been studied in regard to the relation of the dyspnea to the acid-base changes of the blood in paper thirteen (Cullen, Harrison, Calhoun, Wilkins and Timms (1931)) and paper fourteen (Calhoun, Cullen, Harrison, Wilkins and Tims (1931)) These studies showed that neither of these types of dyspnea is due to chemical changes in the arterial or venous blood, or to diminished cerebral blood flow

The respiratory center may be influenced in at least three general ways (a) By chemical changes in the blood, (b) by impulses from the cerebral cortex, (c) by reflexes Having demonstrated that alterations of the first of these influences cannot explain either orthopnea or dyspnea on exertion, and considering it extremely unlikely that any type of dyspnea except that observed in hysteria and allied states can be of psychic origin, we have been led to the conclusion, by exclusion, that the explanation for the types of respiratory distress under discussion was to be sought in reflex disturbances Because of the fact that functional alterations, i e, diminished vital capacity, etc, in the lungs are invariably present in patients with cardiac failure, and since it is well known that respiratory reflexes do arise in the lungs, it was felt that a study of the effects of experimental diminution in vital capacity on breathing was indicated

No attempt is made here to review all of the voluminous literature concerning the reflex regulation of breathing, only a few of the investigations which are of particular importance for the present work will be cited More than a century ago, Legallois (1812) observed that cutting the vagus nerves caused marked decrease in rate and increase in depth of breathing Herring and Brauer (1868) showed that distention of the lungs with air caused an expiratory movement These effects, which were absent after cutting the vagus nerves, were interpreted as indicating that the rate of breathing was controlled by alternating excitant and inhibitory impulses from the lungs to the respiratory center by the vagus nerves Heymans and Heymans (1927) demonstrated that, in addition to these "mechanical" reflexes from the lungs, there existed also, through the vagi, afferent impulses to the respiratory center from the heart and aorta, and that so long as the vagus nerves were intact respiration could be affected by alterations of the pressure and composition of the blood in the heart and aorta Hertzman and Gesell (1927) also found evidences for reflexes from the heart and aorta, but felt that these were less important and less constant than the mechanical reflexes from the lungs

Moore (1927) showed that vagal afferent respiratory reflexes were not solely dependent on lung motion. He separated the breathing of the two lungs by cannulating the two primary bronchi. If when a lung contained oxygen its bronchus were blocked, gradual acceleration of breathing developed, this being sometimes temporary but usually sustained. Cutting the vagus nerve on the corresponding side led to a prompt slowing of the breathing. These researches were extended by Moore and Harrison in work which has not been published. These authors concluded that atelectasis was an important cause of tachypnea, this opinion being based upon the following evidence:

1. If a lung with intact circulation be filled with oxygen and its bronchus blocked, atelectasis develops and a gradual increase in respiratory rate occurs.

2. If the experiment be repeated with the corresponding pulmonary artery ligated, neither atelectasis nor tachypnea develops.

3. If the lung contains nitrogen and has an intact circulation blocking its bronchus does not cause atelectasis or tachypnea.

4. Cutting either vagus nerve slowed the respiratory rate, when tachypnea had been produced.

Moore and Harrison (unpublished data) interpret these observations as meaning that the atelectatic lung was responsible for sending more or less continuous inspiratory impulses to the respiratory center, whereas the other lung, being the sole functioning lung, underwent inflation more rapidly than normal and soon reached a sufficient degree of distention to release inhibitory (expiratory) impulses to the center. The result was rapid and shallow breathing which was to be regarded as essentially due to incoordination of the vagal reflexes.

Harrison and Moore (1928), in another study, found that the vagal influences on depth and on rate of breathing were often dissociated. If one bronchus were compressed so that the corresponding lung had a considerably smaller respiratory excursion than its fellow, cutting the vagus on the corresponding side had little or no effect on respiratory rate but was always associated with a pronounced increase in respiratory depth. As a result of these and similar observations, Harrison and Moore (1928) suggested that the movement of the lungs was the predominant factor in the reflex control of respiratory rate, whereas some other factor, possibly reflexes from the heart and aorta, was especially important in the reflex control of respiratory depth.

It is to be noted that previous students of the subject have not usually made complete studies of the blood gases and hydrogen ion concentration in their observations on reflex regulation of breathing. Consequently there remains the remote possibility that such alterations as were found might have been due to chemical changes in the blood. Gesell (1927) has emphasized the importance of the blood flow through the respiratory center in the control of respiration and has pointed out that in order to draw conclusions concerning the influence of the blood on the center it is necessary to know the composition of the blood coming *from* as well as that going *to* the brain. In the present study we have analyzed both arterial and cerebral venous blood.

#### METHODS

Dogs were used. Sodium barbital in doses of approximately 0.3 gm per kilo of body weight was given two to three hours before the experiment. Tracheotomy was done, and one femoral artery was exposed. The large occipital venous sinus, which has been described by Pilcher (1930) and which drains most of the blood from the posterior portions of

the brain stem, was exposed. Throughout the course of the experiment the animal breathed into a Benedict spirometer, which was filled with oxygen, the carbon dioxide being absorbed in the usual way. Respiratory rates were counted from the tracing except when the breathing was very rapid, in which instance the respirations were counted minute by minute in the course of the observations.

Control observations were made of the respiratory rate and samples of arterial and of occipital venous blood were taken. The vital capacity was then decreased either by (1) introducing air into the thoracic cavity, (2) running Ringer's solution into the lungs through the tracheal cannula, or (3) distending the capillaries of one lung with blood from another dog. The latter procedure was performed as follows.

Before starting the experiment the left fourth or fifth rib was resected, the pleura opened, and artificial respiration instituted. A small glass cannula connected to a small bore rubber tube was then tied into the left pulmonary artery, the open end of the cannula pointing toward the lung. The left pulmonary artery proximal to the cannula was ligated. The left pulmonary veins were then tied. The chest was closed around the rubber tube, care being taken to expel all possible air from the pleural cavity and to distend the lungs with the air pump in order to reinflate any portions which had become atelectatic as a result of the operation. Artificial respiration was then discontinued. At least one half hour was allowed for the breathing to become stabilized before the experimental procedure was begun. In a few instances the animals breathed very rapidly after operation and morphine was given to slow the breathing before the control studies were made. By this technique it was possible to produce any desired degree of congestion of one lung at will, as blood could be introduced into the lung and could not escape from it.

It is obvious that although all of these three procedures reduce the vital capacity, it is certain that introduction of fluid either into the alveoli or vascular bed of the lung has additional reflex effects which are not produced by pneumothorax. In this study, however, it is evident that the reduction of the vital capacity is the important common factor and discussion of the other effects will be omitted during the remainder of this paper for the sake of simplicity.

After the vital capacity had been reduced by one of the methods described the breathing became rapid and a second pair of blood samples (arterial and occipital venous) were taken. The vagus nerves were then cut and the entire experimental procedure repeated. The experiments varied somewhat in the exact details, which are shown in the tables.

The blood samples were taken under oil with Luer syringes. Contamination with air did not occur. Approximately six cubic centimeters of blood were expelled under oil into a bottle containing ovalate. This was used for oxygen determination, which was performed on the Van Slyke-



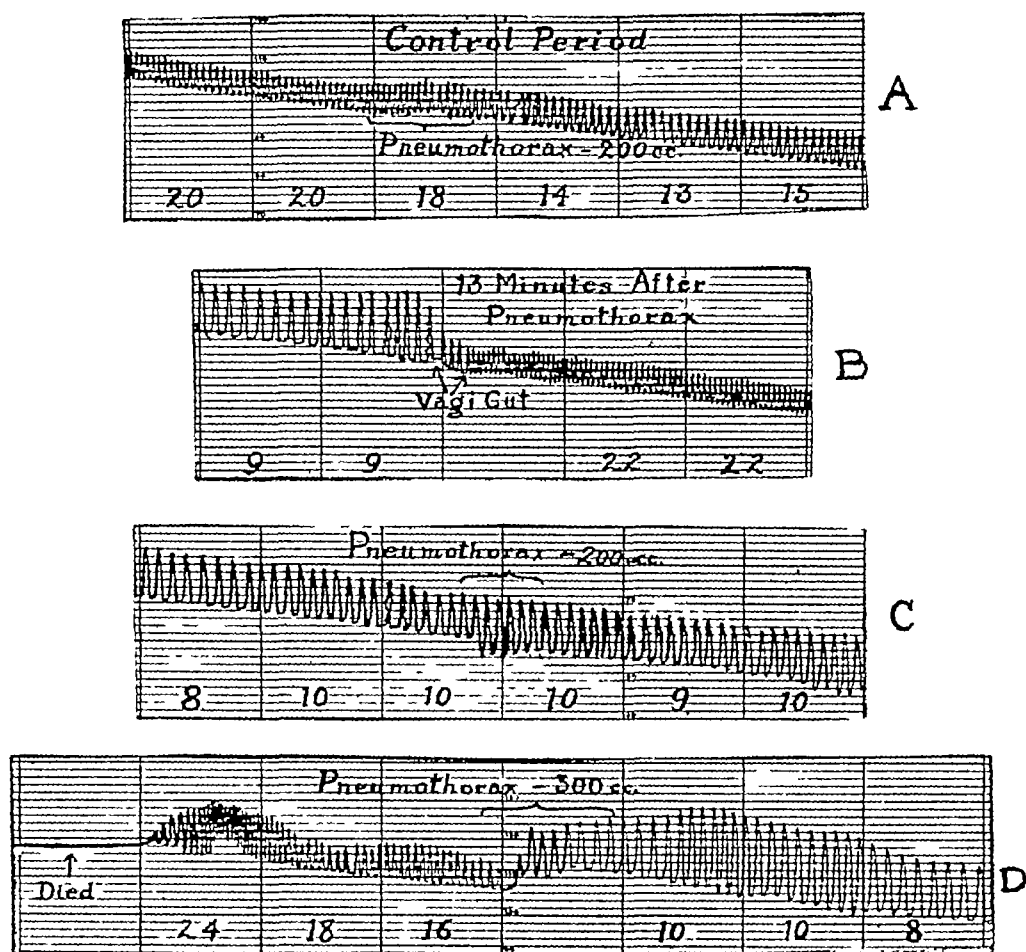


FIG 1 THE CURVES RUN FROM RIGHT TO LEFT

The numbers refer to respiratory rate per minute. Production of pneumothorax when the vagus nerves were intact (A) was followed by a moderate increase in respiratory rate. This persisted until the vagus nerves were cut (B). After vagotomy, pneumothorax did not increase the rate of breathing (C) until sufficient air was introduced to seriously decrease the depth of breathing. Then there was marked tachypnea followed by apnea and death (D). Significant chemical changes in the blood did not occur until the final introduction of air into the chest.

TABLE 1

The effect of reduction of vital capacity on the respiratory rate and on the oxygen saturation and acid base condition of arterial and occipital venous blood

Animal number	Date	Experimental procedure	Respiratory rate per minute	Mean tidal air cc	Ventilation per minute liters	Oxygen content		Arterio-venous difference	Arterial saturation	Carbon dioxide content of serum		pH (colorimetric at 20° C.)		Carbon dioxide pressure	
						Arterial	Occipital venous			Arterial	Occipital venous	Arterial	Occipital venous	Arterial	Occipital venous
1	12/2/30	Control				red smears per cent	red smears per cent			red smears per cent	red smears per cent				
		After pneumothorax 200 cc.	31	91	2 81										
		After pneumothorax 400 cc	42 56	65 38	2 69 2 14										
2	12/15/30	Control	32	97	3 11	11 51	9 72	1 79	94 2	68 1		7 24	7 20	68.3	
		20 minutes after pneumothorax 600 cc.	40	85	3 40	12 71	11 99	0 72	104 0	59 7		7 24	7 27	60 1	
		40 minutes after pneumothorax 600 cc	55	78	4 28	9 36	8 88	0 48	76 5			7 29	7 27		
3	1/6/31	Control	26	124	3 24	17 80	15 14	2 66	103 4	55 0	56 2	7 58	7 49	26 3	32 8
		After pneumothorax 450 cc	38	114	4 34	16 46	15 02	1 44	95 8	54 0	59 0	7 58	7 45	25 8	37 5
		After pneumothorax 650 cc	60	62	3 74	14 75	12 60	2 15	85 8	53 8	59 2	7 57	7 49	26 3	34 6
		After pneumothorax 350 cc	81	73	5 80	16 34	14 53	1 81	95 0	58 1	59 3	7 46	7 46	36 2	39 0
4	1/13/31	Control	24	119	2 86	16 56	14 88	1 68	102 2	53 8	54 8	7 47	7 47	32 9	33 5
		After pneumothorax 200 cc	30	120	3 60	15 84	13 20	2 64	97 7	52 8	54 1	7 46	7 47	32 9	33 0
		After pneumothorax 500 cc	41	102	4 17	16 20	13 80	2 40	100 0	51 8	55 7	7 43	7 42	34 4	37 9
		After pneumothorax 800 cc	64	34	3 45	15 72	13 80	1 92	97 0	53 3	56 3	7 45	7 39	33 9	40 9
		After pneumothorax 1000 cc.	28	62	1 74	6 72	5 76	0 96	41 5	68 6	68 1	7 22	7 27	72 0	64 2
5	1/13/31	Control—vagi intact	4 5	104	0 47	19 68	18 36	1 32	98 4	70 8	68 1	7 24	7 24	71 3	68 3
		After cutting vagi	5 0	217	1 09	18 48	18 12	0 36	92 4	61 5	66 3	7 33	7 33	50 9	62 5
		After pneumothorax 400 cc.	4 0	176	0 70	17 88	17 88	0	89 4	68 5	68 0	7 26	7 26	66 4	61 2
		After pneumothorax 600 cc. (5 minutes after respiration ceased)	0	0	0	1 56	1 08	0 48	8 9	73 8	72 4	<7 10	<7 10	100 0	98 1

TABLE 1 (continued)

Animal number	Date	Experimental procedure	Respiratory rate per minute	Mean tidal air cc	Ventilation per minute liters	Oxygen content		Arterio-venous difference	Arterial saturation	Carbon dioxide content of serum		pH (colorimetric at 20° C.)		Carbon dioxide pressure	
						Arterial	Occipital venous			Arterial	Occipital venous	Arterial	Occipital venous	Arterial	Occipital venous
6	1/16/31	Control—vagi intact After pneumothorax, 300 cc After cutting vagi After additional pneumothorax, 300 cc (Total 600 cc) After additional pneumothorax, 800 cc (Total 1400 cc)	3	265	0.79	vol- umes per cent	vol- umes per cent	0.71	100.0	71.9	72.1	7.34	7.32	58.1	61.1
			17	166	2.82	18.71	18.00	1.68	101.9	69.0	69.8	7.32	7.34	58.4	56.8
			3	433	1.30	19.08	17.40	0.84	102.6	68.8	70.2	7.35	7.33	54.4	58.1
			3	412	1.24	19.20	18.36	1.56	103.3	71.1		7.28		65.4	
			5	170	0.85	19.31	17.75	1.80	45.5	77.4	76.7	<7.10	<7.10	104.8	103.7
7	1/19/31	Control—vagi intact After pneumothorax, 300 cc After cutting vagi After additional pneumothorax, 300 cc (Total 600 cc) After additional pneumothorax, 300 cc (Total 900 cc)	13	172	2.24	17.32	15.56	2.06	100.0	63.7	67.1	7.51	7.49	35.5	39.2
			21	120	2.52	17.32	15.02	2.30	100.0	61.7	67.4	7.49	7.50	21.1	34.4
			9	278	2.50	16.95	13.08	2.87	98.0	61.7	67.5	7.73	7.55	25.2	36.9
			9	238	2.14	14.55	12.96	1.59	84.1	61.7	67.5	7.65	7.52	21.5	
			29	186	5.38	14.90	5.46	9.44	86.1	58.8		7.70	7.53		
8	1/23/31	Control After introducing 200 cc Ringer's solution into lungs After introducing additional 100 cc Ringer's solution After cutting vagi	7	222	1.55	17.55	16.22	1.33	98.1	60.4	60.1	7.37	7.37	45.9	45.7
			20	119	2.38	16.00	14.52	1.48	89.3	59.9	59.4	7.41	7.37	41.7	45.2
			26	83	2.16	16.00	14.52	1.48	89.3	61.6	63.8	7.34	7.31	49.9	55.6
			4	212	0.85	16.10	13.56	2.54	89.9	63.0	64.2	7.23	7.23	64.7	66.0
			19	119	2.26	14.60	12.65	1.95	94.6	63.5	63.9	7.36	7.31	49.3	55.4
9	1/26/31	Control After introducing 100 cc Ringer's solution into lungs After cutting vagi After additional 100 cc Ringer's solution in lungs After 100 cc N/10 lactic acid given intravenously	35	86	3.01	14.24	12.53	1.71	92.2	63.7	65.5	7.38	7.36	47.4	50.8
			10	280	2.80	13.98	8.90	5.08	90.6	57.3	61.7	7.55	7.47	29.2	37.2
			13	166	2.16	12.54	7.43	5.11	81.2	57.8	59.9	7.38	7.36	4.31	46.5
			17	249	4.23	12.04	7.31	4.93	77.9	42.4	51.6	7.36	7.31	32.9	44.7

TABLE 1 (continued)

Animal number	Date	Experimental procedure	Respiratory rate	Mean tidal air	Ventilation per minute	Oxygen content		Arterio-venous difference	Arterial saturation	Carbon dioxide content of serum		pH (colorimetric at 20° C.)		Carbon dioxide pressure	
						Arterial	Occipital venous			Arterial	Occipital venous	Arterial	Occipital venous	Arterial	Occipital venous
12	2/2/31	Control After introducing 100 cc. blood into left pulmonary artery After cutting vagi After introducing additional 100 cc. blood into left pulmonary artery	per minute	cc	liters	tol. water per cent	tol. water per cent	tol. water per cent	per cent	tol. water per cent	tol. water per cent			mm Hg	mm Hg
			41	145	5.94	24.34	20.48	3.86	99.6	55.0	57.8	7.16	7.20	65.3	63.3
			73	42	3.07	24.00	19.88	4.12	99.2	57.6	60.8	7.23	7.21	59.2	65.2
			4	358	1.43	24.22	10.42	13.80	99.0	58.0	65.0	7.20	7.18	63.6	74.5
13	2/2/31	Control After introducing 100 cc blood into left pulmonary artery	4	322	1.29					61.2	70.0	7.22	7.19	64.2	78.4
			6	125	0.75	21.40			103.4	71.9		7.18		82.4	
14	2/5/31	Control After introducing 50 cc blood into left pulmonary artery After cutting vagi After introducing additional 50 cc. blood into left pulmonary artery	35	75	2.61	17.06			82.4	74.2		7.11		98.8	
			48	39	1.87	16.95	15.00	1.95	98.6	59.1	61.1	7.28	7.38	54.7	56.6
			62	29	1.80	13.32	12.85	0.47	77.5	62.8	63.5	7.12	7.12	81.4	82.2
			10	79	0.79	7.52	6.42	1.10	43.7	62.2	63.1	7.12	7.12	80.6	81.7
16	2/10/31	Control After introducing 200 cc blood into left pulmonary artery After cutting vagi After introducing additional 100 cc blood into left pulmonary artery	10	41	0.41	5.82	4.00	1.82	33.8	64.4	64.9	7.05	7.05	97.0	97.8
			18	104	1.87	21.80	20.58	1.22	91.9	64.6	66.8	7.31	7.29	56.0	60.3
			23	93	2.15	23.00	19.72	3.28	98.0	65.0	66.2	7.26	7.26	62.9	64.0
			24	510	1.22	14.77	14.05	0.72	62.9	72.9	71.0	7.18	7.22	83.5	74.6
			23	544	1.25					70.3	69.4	7.24	7.24	70.8	69.9



ence on respiratory depth may be present when there is no influence on respiratory rate

Vagotomy usually caused decrease in minute ventilation, although several experiments were exceptional in this regard. After the vagi had been cut, decrease in vital capacity caused increase in the respiratory rate in only two of seven experiments and in one of these the degree of increase

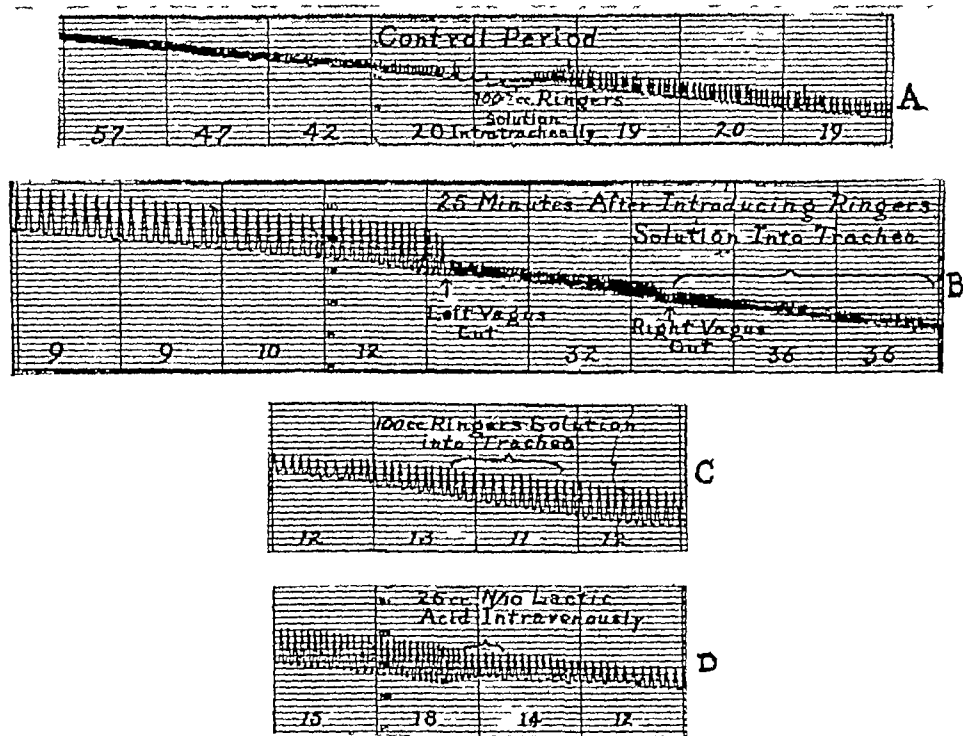


FIG 2 THE CURVES RUN FROM RIGHT TO LEFT

The introduction of Ringer's solution into the lungs caused, after a short period of apnea, marked acceleration of breathing when the vagus nerves were intact (A). Cutting the vagi (B) slowed the breathing. After vagotomy, Ringer's solution was again introduced into the lungs but did not cause rapid breathing (C). However, the animal was able to respond to chemical stimuli as was shown by injecting lactic acid (D).

was small. In the remaining five observations the same procedures which invariably caused rapid breathing in animals with intact vagus nerves had no effect on the respiratory rate. (The reasons why two experiments were exceptions to the general rule are given below.) In vagotomized animals, reduction in vital capacity by any of the three methods used was usually followed by diminution in the depth of breathing and in the minute ventilation.

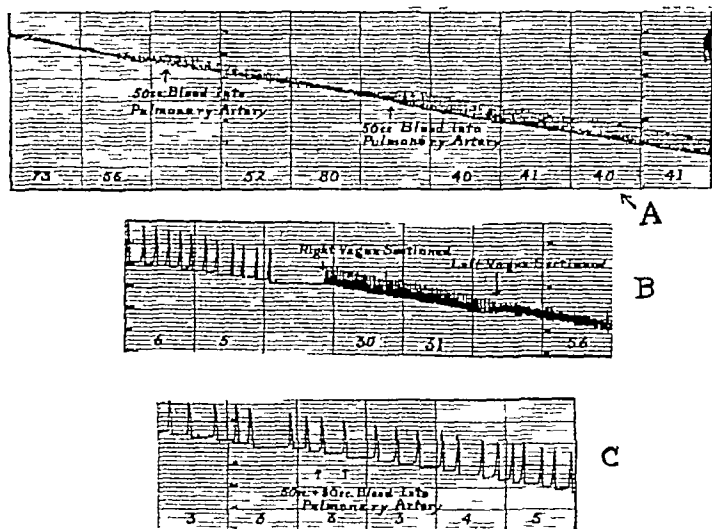


FIG 3 THE CURVES RUN FROM RIGHT TO LEFT

When the vagus nerves were intact distention of the pulmonary capillaries with blood caused tachypnea (A). Cutting the vagi caused slowing of the breathing (B). After vagotomy introducing additional blood into the lung did not increase the rate of breathing (C).

#### *B Changes in the oxygen saturation and acid base condition of the blood*

The initial arterial saturation with oxygen was usually high, as the animals were breathing oxygen. In only two of twelve observations was there significant decrease in the arterial saturation at the time when the respirations first increased in rate. In animals with the vagi intact, progressive diminution in vital capacity caused tachypnea before significant oxygen unsaturation developed. With marked decrease in vital capacity there is a decreased oxygen saturation of arterial blood. The arterial unsaturation therefore could not have been the cause of the tachypnea. Likewise significant alterations in occipital venous oxygen content did not occur from slight reduction in vital capacity. The arteriovenous difference did not undergo any constant changes and hence the rapid breathing cannot be ascribed to diminished cerebral blood flow.

The onset of tachypnea was usually associated with slight diminution of the carbon dioxide content of the blood, both arterial and occipital venous, although in several experiments slight rise in carbon dioxide content was observed. The degree of change was usually not striking. Consistent alterations in pH were not found. In some experiments no change was

noted, in others a decrease, and in still others an increase occurred at the time when rapid breathing began. Likewise, consistent alterations in  $p\text{CO}_2$  did not occur. In the majority of instances decrease in vital capacity caused an initial reduction in carbon dioxide pressure but at times the reverse was found.

Cutting the vagus nerves was usually followed by no marked change in the arterial oxygen saturation. However, in two experiments the marked slowing of respiration was associated with a sharp decrease in arterial saturation. Vagotomy usually altered the occipital venous saturation. The arteriovenous oxygen difference between the blood entering and that leaving the brain was decreased (indicating greater blood flow) three times and increased (indicating lowered blood flow) five times. The differences observed after vagotomy were usually well outside the errors of the method. It appears probable, therefore, that cutting the vagus nerves may be followed either by increase or decrease in cerebral blood flow.

The carbon dioxide content and the pH of the blood were not altered in any constant direction by vagotomy. When the respiratory rate before vagotomy was extremely slow, cutting the vagus nerves resulted in greater ventilation and was likely to be followed by diminution in the carbon dioxide tension and content of the blood and by increase in pH (Experiment 5). In other instances, when vagotomy produced extreme slowing of the respiration, the reverse change occurred (Experiment 16). Often, cutting the vagi produced no significant change in the carbon dioxide content or hydrogen ion concentration, despite marked changes in the rate and character of the breathing.

In the majority of instances the same procedures which produced no consistent changes in dogs with intact vagi were followed by changes in the direction of carbon dioxide acidosis in vagotomized dogs. In the latter animals diminution of vital capacity was as a rule followed by increase in carbon dioxide content, decrease in pH and marked increase in carbon dioxide tension of the serum.

Taken as a whole, such chemical changes as were found in the blood were clearly the results and not the causes of the changes observed in ventilation. This conclusion is the same as that arrived at in our studies on ventilation and blood composition in patients with cardiac disease.

*C The effect of changes of oxygen saturation and acid-base condition of the blood on breathing*

In a number of the experiments which have been mentioned changes in the composition of the blood were found. These were usually slight and it was not believed that changes of such small magnitude could account for the alterations observed in breathing. However, in order to be more certain of this point, experiments were undertaken to determine

the sensitivity of dogs anesthetized in the same manner to changes in the gases and pH of the blood. The data are shown in Table 2 and Figures 4 and 5.

Anoxemia was produced by allowing the animals to rebreathe air from a Benedict spirometer, the carbon dioxide being absorbed. In each instance increase in ventilation resulted, this being due either to increase

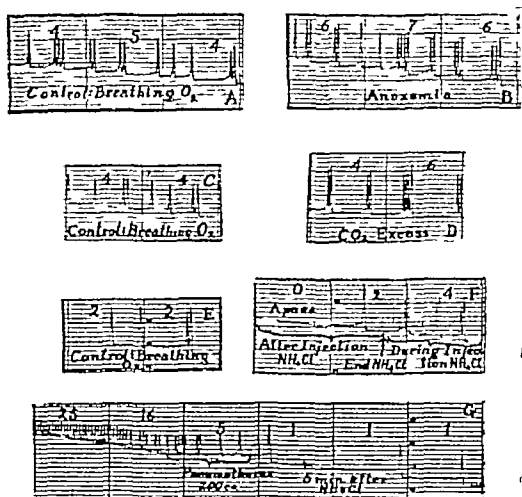


FIG. 4. THE CURVES RUN FROM RIGHT TO LEFT

The vagus nerves were intact throughout the experiment. Anoxemia (B) caused increase in rate and depth as compared to the control period (A). Carbon dioxide excess (D) caused increased depth and slight increase in rate of breathing as compared to the control period (C). Intravenous injection of ammonium chloride (F) caused increase in respiratory rate and depth as compared to the breathing before the injection (E). Following the injection of ammonium chloride apnea developed (F). This was followed by extremely slow breathing (G). Pneumothorax caused prompt and marked increase in the respiratory rate with diminution in depth (G). The experiment illustrates the greater sensitivity of the respiratory rate to changes in the vital capacity (reflex stimuli) than to change in the composition of the blood (chemical stimuli).

in rate, increase in depth, or both. In no instance did anoxemia result in extreme tachypnea. It is therefore clear that the changes in respiratory rate which occurred in the previous experiments when the vital capacity was reduced could not have been due to the slight degrees of anoxemia which occasionally developed.

It appears from Table 2 that, in order to double the minute ventilation the arterial saturation must be reduced to sixty per cent or less. Oxygen

# Vagi Intact

# Vagi Cut

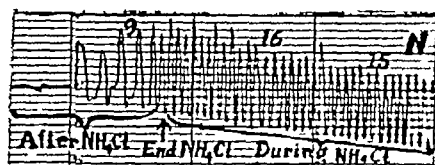
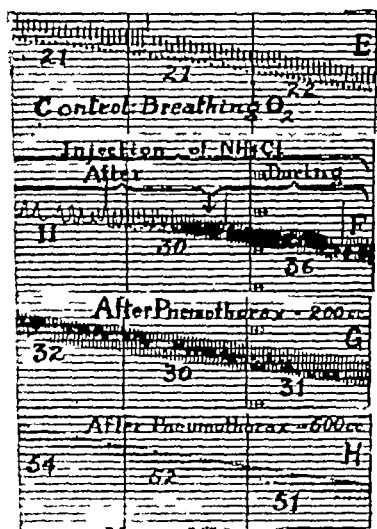
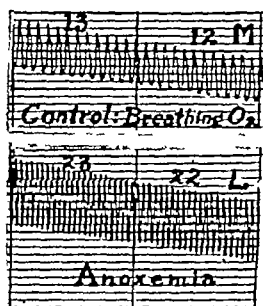
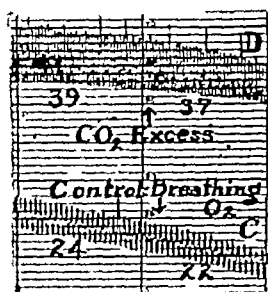
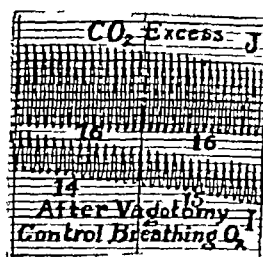
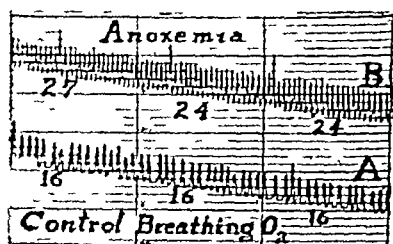


FIG 5 THE CURVES RUN FROM RIGHT TO LEFT

Oxygen lack (B) caused moderate increase in respiratory rate and slight increase in respiratory depth. Carbon dioxide excess (D) caused moderate increase in rate and marked increase in depth of breathing. Intravenous administration of ammonium chloride (F) caused increase in respiratory rate with no distinct change in depth. Following the injection the rate of breathing diminished markedly (F). A small amount of air in the pleural cavity caused moderate increase in respiratory depth (G) and further pneumothorax caused extreme tachypnea (H). Cutting the vagus nerves diminished the rate and increased the depth of breathing (I). After vagotomy carbon dioxide excess (J) produced marked increase in depth and slight increase in rate, oxygen lack caused increase in both rate and depth (L), whereas ammonium chloride caused hyperpnea followed by apnea (N). The response to chemical changes is similar whether the vagus nerves are cut or intact, whereas the response to diminished vital capacity is dependent on the vagus nerves.

TABLE 2

The effect on respiration of changes in oxygenation and acid base condition of the arterial blood of dogs in relation to vagotomy

Animal number	Experimental procedure	O <sub>2</sub> content	O <sub>2</sub> saturation	CO <sub>2</sub> content of serum	pH of serum	CO <sub>2</sub> tension of serum	Respiratory rate	Mean tidal air	Ventilation per minute	Remarks
		vol.umes per cent	per cent	vol.umes per cent		mm. Hg	per minute	cc	liters	
17	Breathing O <sub>2</sub> Control	19.32	100.6	48.2	7.55	24.6	33	87	2.87	Vagi intact throughout experiment
	Rebreathing air (Anoxemia)	15.60	81.3	41.4	7.75	13.5	38	120	4.57	
	Breathing O <sub>2</sub> Control	19.20	100.0	45.5	7.60	20.8	18	114	2.03	
	Breathing O <sub>2</sub> -CO <sub>2</sub> not absorbed CO <sub>2</sub> excess	19.91	103.7	52.3	7.48	31.2	25	166	4.15	
	Breathing O <sub>2</sub> Control	19.32	100.6	47.6	7.52	25.9	21	124	2.61	
	During injection of 1.0 gram NH <sub>4</sub> Cl in 1 minute						43	120	5.16	
	3 minutes after NH <sub>4</sub> Cl						1	210	5.16	
	During injection of additional 1.0 gram NH <sub>4</sub> Cl in 1 minute									
	2 minutes after NH <sub>4</sub> Cl	1.65	92.0	22.5	7.30	20.0	48	145	6.96	
							1	180	0.18	
18	Breathing O <sub>2</sub> Control	20.40	97.6				5	270	1.35	Vagi intact throughout experiment
	Rebreathing air (Anoxemia)	14.53	69.6	65.0	7.60	29.8	6	353	2.11	
	Breathing O <sub>2</sub> Control	21.60	103.2	69.5	7.48	41.4	4	290	1.16	
	Breathing O <sub>2</sub> -CO <sub>2</sub> not absorbed CO <sub>2</sub> excess	21.60	103.2	72.8	7.30	64.2	5	353	1.77	
	Breathing O <sub>2</sub> Control	20.65	98.9	71.8	7.34	58.2	2	360	0.72	
	During injection of 1.0 gram NH <sub>4</sub> Cl in 4 minutes						5	315	1.57	
	After injection of NH <sub>4</sub> Cl						0.5	300	0.15	
	After pneumothorax 200 cc.	21.0	100.4	56.0	7.40	39.7	25	104	2.59	
19	Breathing O <sub>2</sub> Control	18.15	98.1	57.6	7.50	32.9	20	155	3.10	Vagi intact
	Rebreathing air (Anoxemia)	8.28	44.8	51.4	7.58	24.6	39	155	6.06	
	Breathing O <sub>2</sub> Control	19.10	103.2	55.4	7.60	25.3	25	176	4.40	
	Breathing O <sub>2</sub> -CO <sub>2</sub> not absorbed CO <sub>2</sub> excess	18.60	100.5	57.5	7.55	29.4	39	270	8.10	
	Breathing O <sub>2</sub> Control	18.36	99.3	53.2	7.63	22.7	22	135	2.97	
	During injection of NH <sub>4</sub> Cl after 0.3 gram in 1 minute	18.74	101.2	48.3	7.55	24.7	32	186	5.31	
	Immediately after injection of 1.6 gram NH <sub>4</sub> Cl in 7 minutes									
	2 minutes after injection of NH <sub>4</sub> Cl	17.65	95.5	37.5	7.25	37.0	36	128	4.62	
	Before pneumothorax						6	120	0.72	
	After pneumothorax 700 cc.	16.70	90.3	34.4	7.50	19.6	5	128	0.64	
							58	93	5.42	
	After cutting vagi Breathing O <sub>2</sub> Control						14	288	3.20	
	Breathing O <sub>2</sub> -CO <sub>2</sub> not absorbed CO <sub>2</sub> excess						16	425	6.80	
	Breathing O <sub>2</sub> Control						12	276	3.31	
	Rebreathing air (Anoxemia)						28	363	10.17	
	Breathing O <sub>2</sub> Control						13	249	3.23	
	During injection of NH <sub>4</sub> Cl 1.3 gram in 7 minutes						15	456	6.85	
	1 minute after NH <sub>4</sub> Cl						1	360	0.36	
	5 minutes after NH <sub>4</sub> Cl						2	384	0.77	
	After pneumothorax						3	290	0.87	
20	Breathing O <sub>2</sub> Control	19.58	102.5	48.9	7.38	36.4	13	207	2.70	Vagi intact
	Rebreathing air (Anoxemia)	13.20	69.1	45.5	7.65	18.6	29	187	5.42	
	Breathing O <sub>2</sub> Control	19.44	101.7	47.8	7.42	32.5	7	320	2.23	
	Breathing O <sub>2</sub> -CO <sub>2</sub> not absorbed CO <sub>2</sub> excess			51.6	7.31	44.7	9	477	4.30	
	Breathing O <sub>2</sub> Control			50.9	7.46	31.7	10	238	2.38	
	During injection of NH <sub>4</sub> Cl 1.2 gram in 6 minutes			46.0	7.32	39.0	10	274	2.74	
	Immediately after NH <sub>4</sub> Cl						3	435	1.30	
	Before pneumothorax						3	435	1.30	
	After pneumothorax 200 cc.						17	207	3.52	
	After vagotomy Breathing O <sub>2</sub> Control	15.60	81.7	44.7	7.21	48.0	4	518	2.07	
	Rebreathing air (Anoxemia)	11.64	60.5	40.0	7.58	19.1	6	621	3.73	
	Breathing O <sub>2</sub> Control	18.46	96.8	45.8	7.31	39.8	3	538	1.62	
	Breathing O <sub>2</sub> -CO <sub>2</sub> not absorbed CO <sub>2</sub> excess			47.9	7.26	49.5	3	591	1.77	
	Breathing O <sub>2</sub> Control						4	497	1.99	
	During injection of NH <sub>4</sub> Cl 1.2 gram in 5 minutes			40.4	7.21	43.4	6	435	2.61	
	1 minute after NH <sub>4</sub> Cl						1	674	0.67	
	5 minutes after NH <sub>4</sub> Cl									
	Before pneumothorax						4	436	1.74	
	After pneumothorax 200 cc.						6	290	1.74	

lack was associated with striking decrease in carbon dioxide tension and marked rise in pH. These changes were, of course, the results of the increased ventilation.

*Carbon dioxide excess* was produced by allowing the animal to rebreathe oxygen from a Benedict spirometer, the soda lime container having been removed. The breathing was more sensitive to carbon dioxide excess than to oxygen lack, the response being not only quantitatively but also qualitatively dissimilar. The reaction to increased carbon dioxide in the inspired air was characterized by relatively greater increase in depth and relatively less increase in rate than was the response to anoxemia.

Although the animals were more sensitive to excess of carbon dioxide than to lack of oxygen, their sensitivity to the former stimulus was much less than has been generally believed. Haldane and Priestley (1905) stated that a rise of one millimeter in the carbon dioxide tension of the alveolar air was sufficient to double the ventilation. Such a change would correspond to a decrease of 0.01 to 0.02 in pH. In our animals the smallest increase in  $p\text{CO}_2$  found at a time when the ventilation was approximately doubled was 5 mm, and the smallest decrease in pH was 0.04. The average change in these values was much greater, being 11 mm and 0.12 pH respectively when the ventilation was increased by approximately 100 per cent. One might raise the objection that our studies were performed on anesthetized (barbital) dogs, whereas the observations of others have been made on normal men. On the other hand, studies on the alveolar air alone can give at most only incomplete and possibly unreliable information about the blood, even assuming that one gets a true alveolar sample of air. Furthermore, it has already been shown (Cullen and Earle (1929)) that changes of considerable degree may occur in the pH and  $p\text{CO}_2$  of normal individuals during the course of the day, with no striking changes in ventilation, and further (Study XIII of this series) that marked changes in ventilation may occur in normal men with no change, or even shifts toward alkalinity, in the pH and  $p\text{CO}_2$ . For these reasons we agree with Gesell (1927) that the doctrine of the extreme sensitivity of the respiratory center to changes in the blood composition is incorrect.

The pH of the blood was reduced by injecting ammonium chloride. In each experiment characteristic changes in breathing were noted. During the injection the ventilation increased in each instance, this change being due either to increase in depth or rate or both. The change in depth was less striking and less constant than was found when carbon dioxide was breathed, the response to ammonium chloride being similar to that produced by oxygen lack. Within one to three minutes after the injection the breathing decreased in frequency and apnea sometimes resulted. In one animal this was fatal. The pH was markedly diminished by ammonium chloride in each instance, the degree of decrease varying

from 0.14 to 0.30. The carbon dioxide tension was usually elevated when the blood was drawn while the salt was being injected or immediately after. Studies were not made of the blood during the apneic period following the injection but it is considered probable that at this time the  $p\text{CO}_2$  was much diminished.

In several experiments observations were made, during the apneic period following the administration of ammonium chloride, of the effect of diminishing the vital capacity by pneumothorax. In each instance a prompt increase in respiratory rate was observed, provided the vagi were intact (Fig. 4).

*The effect of changes in the acid base condition of the blood on the breathing of vagotomized dogs* was studied in two animals (Table 2, animals 19 and 20). It is seen that oxygen lack, carbon dioxide excess and ammonium chloride were always followed by increased minute ventilation, and often followed by increased respiratory rate. Possibly the vagotomized dogs were somewhat less responsive as regards ventilation than were the animals with intact vagi, but our data are not complete enough to prove this. The work of Heymans and Heymans (1927), indicating that not only the respiratory center but the peripheral vagal afferent fibers to the center are also stimulated by chemical changes in the blood, would suggest such a conclusion.

It should be emphasized again that the dogs responded differently to excess carbon dioxide than to oxygen lack or ammonium chloride. According to the Haldane (1922) theory of respiratory control, one would expect a dissimilar reaction to the former two stimuli but one would expect a similar response to acidosis, whether produced by volatile or non-volatile acid. However, the effect of ammonium chloride was somewhat similar to that of oxygen lack, and was rather unlike that of carbon dioxide excess. This fact seems to confirm the idea of Hooker, Wilson, and Connett (1917), and Scott (1918), that carbon dioxide has a more or less specific effect as well as an acid effect. This specific effect is probably as Jacobs (1920) has suggested, a function of the great diffusibility of  $\text{CO}_2$ . Further observations along this line are being made.

As a result of these observations, showing the relative insensitivity of the ventilation to changes in blood composition, and more especially the relative insensitivity of the rate of breathing to such changes, it seems clear that the marked increases in respiratory rate which were produced by diminishing the vital capacity and which were associated with no change or only slight alterations in the blood composition could not have been due to changes in the blood. These observations, as well as those concerning the effect of vagotomy seem to show clearly that tachypnea resulting from diminished vital capacity is due to afferent vagal reflexes.



## DISCUSSION

In the preceding paper of this series (Calhoun, Cullen, Harrison, Wilkins, and Tims (1931)), studies were made of orthopnea. It was found, as Christie and Beams (1922) had previously reported, that on assuming the recumbent posture the patient with orthopnea has a significant reduction of vital capacity. Hence, in the recumbent posture the tidal air became a greater fraction of the vital capacity and this was believed to be one important cause of the dyspnea in the recumbent position. But it was also found that patients with severe orthopnea often breathed more rapidly in the recumbent than in the sitting posture, a paradoxical effect, being the opposite reaction to that usually observed in normal persons who often breathe more slowly in the recumbent position. It was evident that this increase in the rate of breathing in a person whose respiratory muscles were already performing more than the normal amount of work was an important factor in the production of further subjective respiratory distress. In the study referred to, the cause of this increase in the rate of breathing was not found. The present study indicates clearly that decrease in vital capacity can, *per se*, produce rapid breathing by reflexes from the lungs. The mechanism of orthopnea therefore seems to be clear.

The patient with congestive heart failure of moderate degree has a considerable decrease in his vital capacity, even when sitting. Although his respiratory rate is likely to be somewhat greater than normal his tidal air is usually a larger fraction of his vital capacity than is the case in a normal individual. This in itself means a greater respiratory effort per breath. But, due to his decreased vital capacity he has a slight increase in respiratory rate. Hence, he has to make more than the normal number of muscular efforts per minute to breathe. Because of the great reserve power of the respiratory muscles he may have only slight or even no respiratory distress.

On lying down his vital capacity is, because of increased volume of blood in the lung and higher diaphragm, diminished still further, by possibly 300 cubic centimeters. To a normal subject such a decrease, being only perhaps eight per cent of the total vital capacity, would make no perceptible difference. But as has been stated, the patient was already unconsciously drawing on his respiratory reserves, and furthermore, this decrease may represent a fifteen per cent (or greater) diminution in his vital capacity. If his minute ventilation is to remain the same he has to either breathe a still larger fraction of his vital capacity at each breath or he has to breathe faster. Actually he does both in many cases. The result is not only an increase in the muscular effort involved for each breath but, because of vagal reflexes, an increase in the number of efforts each minute. He was already using some of the reserve power of his respiratory muscles but not enough to be conscious of the effort involved.

The increase in the muscular effort of breathing becomes, on lying down, sufficient to produce a sensation of fatigue in the respiratory muscles and he feels "short of breath." Other factors, such as the sense of congestion in the head due to the increase in venous pressure on lying down and slight decrease in the aeration of the blood in the recumbent posture, may play some rôle but the essential features in the mechanism of his orthopnea are those described.

At a later stage of his disease, the patient's vital capacity is still less. Even when he sits up it is very low. The result is the constant resting dyspnea, which is such a distressing feature of the terminal stage of cardiac failure in many patients.

Orthopnea occurs not only in cardiac disease but also in certain other conditions, such as severe cases of pneumonia, pleural effusion, marked ascites, pneumothorax, massive collapse of the lung etc. All of these conditions are characterized by diminished vital capacity and by increased respiratory rate. It seems to us likely that in these cases also orthopnea and dyspnea are due, in the main, to decreased vital capacity and consequent reflex tachypnea. Dyspnea in pneumonia has never been satisfactorily explained. Undoubtedly, pain, fever with increased metabolic rate, and anoxemia play a rôle. However, one observes patients with pneumonia who have little or no pain, whose anoxemia has been relieved by oxygen therapy, and who still are breathing rapidly—more rapidly than can be explained by fever alone. It is probable that the rapid breathing in such cases is due to reflexes caused by decrease in vital capacity. This conclusion is somewhat similar to that arrived at by Binger and his co-workers (1925-1927), who in a series of studies on rapid breathing were led to the view that the changes in the lungs themselves were responsible for the tachypnea.

Investigations of the blood have provided satisfactory explanations for the breathing of diabetic and renal acidosis, which are characterized by marked increase in the depth and relatively slight increase in the rate of breathing. Adequate explanations for the shallow rapid breathing of conditions associated with cardiac or pulmonary disorders and hence with decreased vital capacity, have not been furnished by chemical studies of the blood, and hitherto have been lacking. We believe that reflexes from the lungs are the chief cause of dyspnea in such conditions.

The concept of the extreme delicacy of the respiratory response to chemical alterations in the blood must, as has been emphasized by Gesell (1927), be abandoned. From the present and the preceding studies of this series (XIII Cullen et al. XIV Calhoun et al.) it seems evident that the nervous regulatory mechanism is more delicate than the chemical. One can think of the latter as being like the coarse adjusting screw and of the former as being like the fine adjusting screw of a microscope. The sensitivity of the reflex respiratory control is a fact of considerable signifi-

cance Because of it respiration is altered in such a way as to tend to prevent gross chemical changes in the blood The constancy of the "*milieu intérieur*" (Claude Bernard) is thereby maintained

#### SUMMARY

Studies have been made of the respiratory rate and depth, the minute ventilation, and of the oxygen, carbon dioxide and pH of the arterial blood and of the venous blood from the brain of dogs anesthetized with barbital In some experiments artificial reduction of vital capacity was produced either by pneumothorax, by introducing fluid into the lungs through the trachea, or by distending the capillaries of one lung with blood, according to a technique which has been described In other experiments observations were made concerning the sensitivity of the respiration to oxygen lack, carbon dioxide excess and to acidosis produced by the intravenous injection of ammonium chloride The following results were obtained

- 1 Reduction of vital capacity by any of the methods used resulted in rapid breathing, provided the vagus nerves were intact In such experiments chemical changes of the blood were usually either absent or in the direction of increased alkalinity

- 2 In vagotomized dogs diminution in vital capacity was usually not followed by rapid breathing, unless the diminution was of sufficient degree to produce either marked oxygen lack or increased acidity of the blood

- 3 Oxygen lack, produced by rebreathing, caused increased ventilation either by increase in depth, rate or both In order to double the ventilation it was usually necessary that the arterial blood be less than 60 per cent saturated

- 4 Carbon dioxide excess caused marked increase in depth and relatively slight increase in rate of breathing The response of the animals to carbon dioxide excess was quantitatively greater and qualitatively different from that of oxygen lack In order to double the ventilation it was usually necessary to produce a fall of approximately 0.10 in pH and a rise of 10 mm Hg in carbon dioxide tension of the arterial blood

- 5 The effect of the acidosis produced by ammonium chloride on the breathing was unlike that of carbon dioxide excess and rather similar to that of oxygen lack, being characterized by a relatively great increase in rate and only slight increase in depth Following the injection of ammonium chloride apnea sometimes occurred

- 6 Chemical changes in the blood never produced the extreme degree of tachypnea which resulted from diminished vital capacity

- 7 Vagotomized dogs, although insensitive to diminution in vital capacity, reacted with increase in ventilation to chemical changes in the blood

## CONCLUSIONS

From these observations the following conclusions have been drawn

1 Orthopnea and the continuous dyspnea at rest which occurs in the terminal stages of cardiac disease are of reflex origin and dependent on diminished vital capacity

2 It is probable that the rapid breathing found in various diseases of the thoracic organs accompanied by decrease in vital capacity is essentially of reflex origin

3 The reflex mechanism of respiratory control is more sensitive than the chemical mechanism. The respiratory center seems to be much less sensitive to alterations in the composition of the blood than has been generally believed

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## GASTRIC ACIDITY IN NORMAL INDIVIDUALS

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A review of the literature reveals comparatively little information concerning gastric acidity in normal individuals. The available data are based mostly on test meals of the Ewald type. In addition to the technical difficulties in analysis inherent in these methods, they fail to give a maximum stimulation for secretion of gastric juice, as do histamine or histamine plus alcohol. Consequently the average acidities of the available standards are low and the incidence of achlorhydria high. As demonstrated by Gompertz and Vorhaus (1) and Bockus and Bank (2) histamine used in conjunction with the test meal enables one to differentiate true from false achlorhydria. Thus Davies and James (3), studying normal persons over the age of 60, and Vanzant (4) investigating a large series of normals of all ages, have made use of this means of differentiating true from false achlorhydria. Except for the recently published data of Pollard and Bloomfield (5), we have been unable to find any normal gastric acidity standards based on histamine as the gastric secretory stimulant. An objection to their results may be raised, namely, they do not contain figures for the incidence of achlorhydria.

Because of the lack of suitable data concerning the gastric acidity of the normal individual it was deemed advisable to collect our own from the hospital records before proceeding with our study of the gastric secretion in various pathological conditions. We are placing these on record, with the hope that they may be of value to those who use the technique of gastric analysis similar to ours.

### METHOD

The gastric contents were obtained in the morning after a 12 hour fasting period. A gastric tube was introduced through the nose and the fasting contents removed. This was followed by the subcutaneous injection of 0.5 mgm. ergamine acid phosphate (histamine) and the administration through the tube of 50 cc. of 7 per cent alcohol. Samples of gastric contents were drawn at half hour and one hour intervals, all of the contents being removed with the last sample. The volume, and the free and total acidity of each sample were determined, using Töpfer's reagent and phenolphthalein as the indicators in the titration of the respective acidities. In addition, the gross physical characteristics and the presence

of occult blood were recorded. All analyses were made by various members of the resident medical staff.

We have concerned ourselves mainly with the free and total acidity and their correlation with age, sex, red blood cell count and hemoglobin content. In making the tables and charts we used the maximum acidity obtained following stimulation with histamine and alcohol. In the case of the red blood cell count and hemoglobin content, the average values were taken.

The measurements of volume were considered too inaccurate to furnish any useful information.

We did not use the method of continuous withdrawal of gastric contents at 10 minute intervals as advocated by Pollard, Roberts and Bloomfield (6). The use of alcohol plus histamine makes our data incomparable to those of Pollard and Bloomfield (5) based on histamine alone, but our data are more comparable to those of Vanzant (4), based on a test meal of crackers and water, and histamine in cases of achlorhydria. The alcohol test meal has the additional advantage of meeting the criticisms offered by Henning (7) and by Comfort and Osterberg (8), namely, that certain cases fail to secrete free acid with histamine but do so with a test meal.

#### MATERIAL

The records of all patients having had gastric analyses during the years 1928-1930 inclusive were examined. Out of a total of 720 records, 200 were selected as representing normal individuals. All persons suffering from any disease which might possibly affect gastric secretion were excluded from this series of cases. In addition, the absence of severe gastro-intestinal symptoms, roentgenological examination of the gastro-intestinal tract and Graham test and a relatively negative physical examination were required before a patient was considered normal.<sup>1</sup>

Most of the 200 cases were in the age groups of 20-69, fairly uniformly distributed. There were 5 under 20 years of age, and 5 over 70. There were 90 males and 110 females. The results for free and total acidity in relation to age are tabulated in Tables 1 and 2. The normal acidity range was considered to be 20-70 cc. for free acid and 10 points higher for total acid.

The average free acidity was 40.4 cc. of 0.1 normal acid per 100 cc. There was a fluctuation in the age groups between 36.8 cc. and 57.8 cc., but no definite relation to age. The total acidity ranged approximately 10 points higher, the average being 50.1 cc. On calculating the coefficients of correlation between age and gastric acidity, we found the results to be

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<sup>1</sup> There were a number of doubtful cases which were examined very carefully with the help of Dr. Chester M. Jones before they were included as normals or rejected.

TABLE 1

*Distribution by age of the free acidity of 200 normal subjects*

Cc. 0.1 N HCl <i>per 100 cc.</i>	Age (Years)							Total
	-20	20-29	30-39	40-49	50-59	60-69	70+	
	<i>cases</i>	<i>cases</i>	<i>cases</i>	<i>cases</i>	<i>cases</i>	<i>cases</i>	<i>cases</i>	<i>cases</i>
0		1	5	4	10	6		26
1-9		1	2	2	4	2	1	12
10-19		2	5	4	5	4	1	21
20-29	1	4	4	3	7	3		22
30-39		2	6	5	1	3		17
40-49	1	4	5	7	2	4		23
50-59	2	3	5	3	5	3		21
60-69		2	2	3	4	5		16
70-79	1	2	1	5	5	3	1	18
80-89		1	5	1	4	2		13
90+		1		3	4	1	2	11
Total	5	23	40	40	51	36	5	200
Average acidity	50.2	43.3	36.8	42.4	38.7	38.8	57.8	
Average of 174 cases with free acid	50.2	45.3	42.1	47.1	48.1	46.6	57.8	

Standard deviation of acidity = 28.4

Standard deviation of age = 14.3

TABLE 2

*Distribution by age of the total acidity of 200 normal subjects*

Cc. 0.1 N HCl <i>per 100 cc.</i>	Age (Years)							Total
	-20	20-29	30-39	40-49	50-59	60-69	70+	
	<i>cases</i>	<i>cases</i>	<i>cases</i>	<i>cases</i>	<i>cases</i>	<i>cases</i>	<i>cases</i>	<i>cases</i>
0-9		1	7	3	8	4		23
10-19		2	1	4	5	5	1	18
20-29		2	5	4	7	3	1	22
30-39	1	3	5	3	4	2		18
40-49	1	2	3	4	4	3		17
50-59	1	4	7	7	3	2		24
60-69	1	3	3	2	1	4		14
70-79			2	5	7	6	1	21
80-89	1	3	3	3	5	2		17
90-99		2	3	3	3	4		15
100+		1	1	2	4	1	2	11
Total	5	23	40	40	51	36	5	200
Average acidity	57.2	53.2	46.7	51.4	48.2	50.4	62.6	

Standard deviation of acidity = 30.7

Standard deviation of age = 14.3



—  $0.015 \pm 0.048$  for free acid and  $+0.009 \pm 0.048$  for total acid, values which are not significant

The incidence of anacidity was 13.0 per cent, of hypoacidity 16.5 per cent, of normal acidity 49.5 per cent and of hyperacidity 21.0 per cent. The results for the incidence of achlorhydria with age are extremely interesting and are represented graphically in Figure 1. The unusual finding was the low incidence of anacidity in the age groups of 65 and over, i.e. 5.3 per cent of the 19 cases in these groups. Taking the data as a whole we find there is no significant correlation between age and incidence of anacidity. However, as the chart illustrates there is a definite

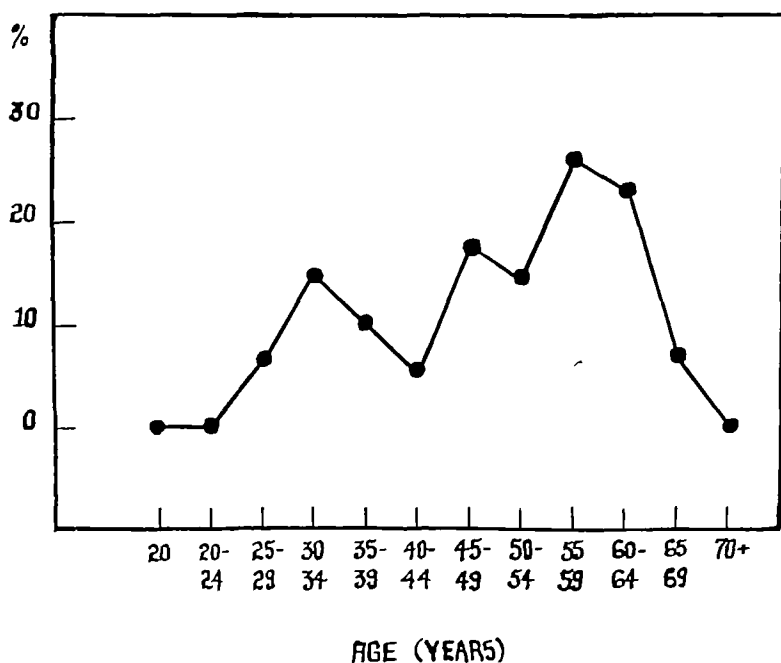


FIG. 1 THE PERCENTAGE INCIDENCE OF ACHLORHYDRIA IN NORMAL PEOPLE IN DIFFERENT AGE GROUPS

increase in the incidence up to the age of 65. Of the 28 cases under 30 years of age 3.6 per cent showed achlorhydria, whereas 24.4 per cent of the 45 cases belonging in the age groups of 55–64 revealed this same finding. The coefficient of correlation between the incidence of achlorhydria and age (up to 65 years) is  $+0.85 \pm 0.062$ , a very significant one. The decrease in incidence after 65 years is difficult to explain. Vanzant (4) makes the interesting suggestion that the mortality rate may be higher in persons with achlorhydria than in those with free acid in the stomach. These older people also showed a tendency to hypersecretion. For example, the incidence of hyperacidity in the age groups under 65 was 19.3 per cent, with relatively slight fluctuation in the different age groups, whereas over 65 it was 36.8 per cent.

The preceding results are not in accord with the data of other investigators. For example, Bloomfield and Keefer (9), using an alcohol test meal, and Pollard and Bloomfield (5), using histamine, found a definite correlation between acid secretion and age. The former workers also noted an increased incidence of achlorhydria with advancing age. Our results agree qualitatively with those of Vanzant (4).

As indicated in another communication (10) there is a striking difference in the gastric secretion of the sexes. Figure 2 shows that the average secretion of free acid by age was usually higher in the male than in the female, but after the age of 50 the tendency was towards equal values. The average free acidity for the male was  $44.7 \pm 1.91$  cc. and for the fe

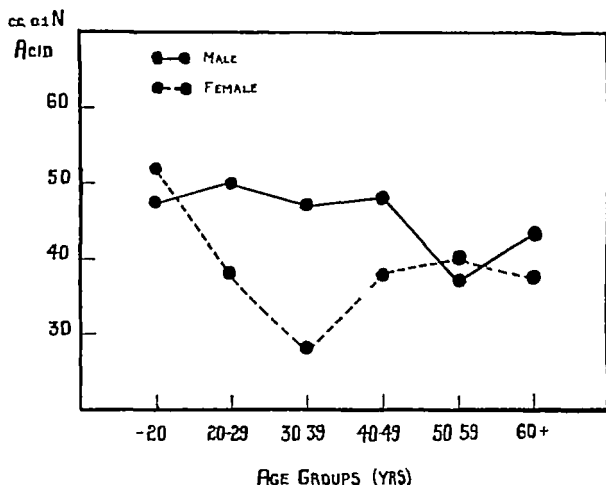


FIG 2 THE AVERAGE SECRETION OF FREE ACID OF NORMAL MALE AND FEMALE PATIENTS ACCORDING TO AGE

male  $36.8 \pm 1.86$  cc, a difference of  $7.9 \pm 2.67$  cc. This value is definitely significant because it is about three times its probable error. There was no correlation between acidity and age in each sex. The increase in achlorhydria was present in both sexes, but to a relatively greater degree in the male.

The sex differences may also be demonstrated by comparing the incidence of the various degrees of free acidity.

	Male (90) per cent	Female (110) per cent
Anacidity	10.0	15.4
Hypoauidity	11.1	20.9
Normal acidity	55.6	44.5
Hyperacidity	23.3	19.2

These results indicate that females more frequently have anacidity and hypoacidity, and less often hyperacidity. Mathematically, the odds are 8 to 1 against the occurrence of such results on the basis of chance alone. In Figure 3 the above percentages are compared graphically. The differences appear even greater because the results for the males have been corrected for age distribution, using the females as standard. It should be noted that the results for total acidity ran parallel with those for free acidity and therefore are not recorded.

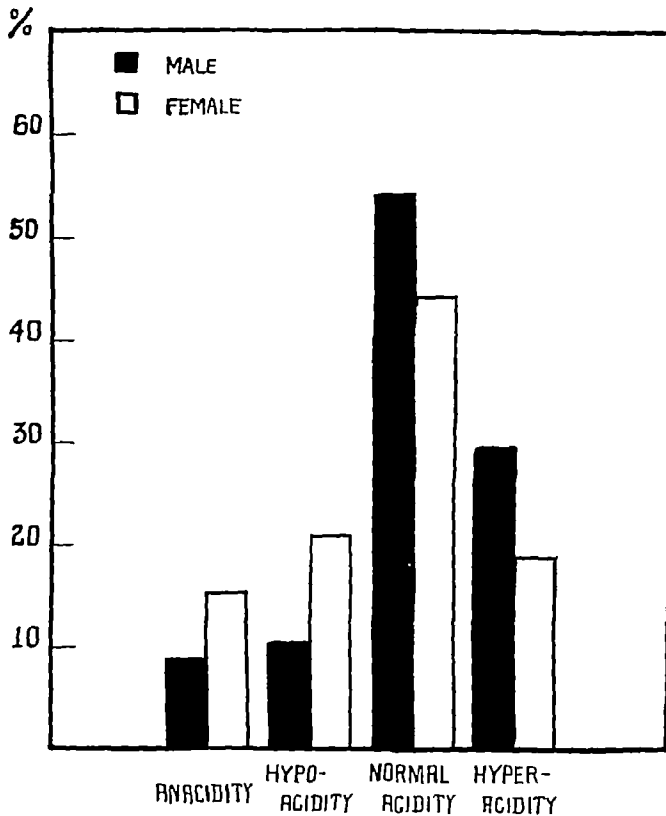


FIG 3 THE PERCENTAGE INCIDENCE OF THE VARIOUS DEGREES OF ACIDITY IN NORMAL MALE AND FEMALE PATIENTS

The results are corrected for age distribution of males as compared to the females

An attempt was made to correlate the gastric acidity with the red blood cell count and hemoglobin content. The distribution data for free acidity are given in Tables 3 and 4. It is evident from Figure 4, that the red blood cell count and hemoglobin content tended to vary directly with the average acidity. For example, the average free acidity for the 22 cases with counts under 4 million cells was 31.4 cc. and for the 21 cases with counts over 5.5 million cells was 53.3 cc., the average free acidity for the 15 cases with hemoglobin values below 60 per cent was 19.7 cc. and

TABLE 3

*Distribution of the free acidity of 199 normal subjects according to level of red blood cell count*

Cc. 0.1 N HCl  per 100 cc.	Red blood cell count (Millions)							Total
	-3.50	3.50-3.99	4.00-4.49	4.50-4.99	5.00-5.49	5.50-5.99	6.00+	
0	cases 2	cases 2	cases 8	cases 7	cases 7	cases	cases	cases 26
1-9	1		4	3	3	1		12
10-19	2	3	7	4	3	2		21
20-29		1	7	9	3	1	1	22
30-39	1	2	2	4	6	1	1	17
40-49	1	1	3	9	6	3		23
50-59		1	4	8	6	2		21
60-69	1	2	4	2	5	2		16
70-79	2		3	5	5	1	1	17
80-89			5	3	2	1	2	13
90+			3	2	4	1	1	11
Total	10	12	50	56	50	15	6	199
Average acidity	32.2	30.8	37.4	38.8	42.8	47.4	68.0	

Standard deviation of acidity = 28.4

Standard deviation of red blood cell count = 0.64

TABLE 4

*Distribution of the free acidity of 198 normal subjects according to hemoglobin levels*

Cc. 0.1 N HCl  per 100 cc.	Hemoglobin (Per cent)						Total
	-50	50-59	60-69	70-79	80-89	90+	
0	cases 3	cases 3	cases 5	cases 4	cases 7	cases 4	cases 26
1-9	1			5	4	2	12
10-19	2	1	3	3	9	3	21
20-29			5	6	6	5	22
30-39	1	1	2	3	8	2	17
40-49	1		6	7	5	4	23
50-59		1	2	9	6	3	21
60-69			4	6	4	2	16
70-79	1		2	3	7	4	17
80-89			1	5	4	2	12
90+			1	3	7		11
Total	9	6	31	54	67	31	198
Average acidity	20.6	18.1	38.3	44.2	42.9	37.7	

Standard deviation of acidity = 28.3

Standard deviation of hemoglobin = 12.5

for the 98 cases with hemoglobin values of 80 per cent or over was 41.3 cc. The coefficient of correlation between the free acidity and red blood cell count is  $+0.195 \pm 0.046$ , or 4.2 times its probable error, between the free acidity and hemoglobin content the coefficient is  $+0.123 \pm 0.047$ , or 2.6 times its probable error. The former is highly significant but the latter only probably so. The corresponding coefficients for total acidity are  $+0.205 \pm 0.046$  and  $+0.141 \pm 0.047$  respectively. These correlations apply to all levels of acidity, a fact which is more clearly demonstrated in Tables 5 and 6. These tables condense and summarize the

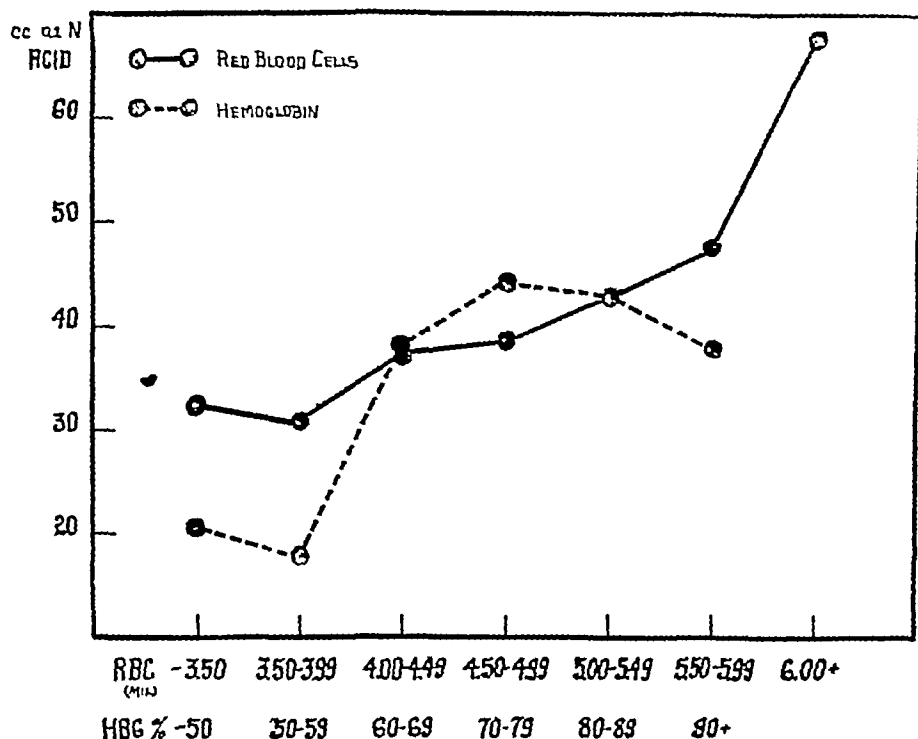


FIG. 4. THE AVERAGE FREE ACIDITY OF NORMAL PEOPLE ACCORDING TO THE LEVELS OF RED BLOOD CELL COUNT AND HEMOGLOBIN CONTENT

TABLE 5

*The percentage incidence of the various degrees of free acidity in relation to red blood cell count*

	Red blood cells (Millions)				
	-4.00	4.00-4.49	4.50-4.99	5.00-5.49	5.50+
	per cent of cases	per cent of cases	per cent of cases	per cent of cases	per cent of cases
Anacidity	18.2	16.0	12.5	14.0	0
Hypoaclidity	27.3	22.0	12.5	12.0	14.3
Normal acidity	45.5	40.0	57.1	52.0	52.4
Hyperacidity	9.1	22.0	17.9	22.0	33.3

TABLE 6

*The percentage incidence of the various degrees of free acidity in relation to hemoglobin*

	Hemoglobin (Per cent)				
	-60	60-69	70-79	80-89	90+
	<i>per cent of cases</i>	<i>per cent of cases</i>	<i>per cent of cases</i>	<i>per cent of cases</i>	<i>per cent of cases</i>
Anacidity	40.0	16.1	7.4	10.4	12.9
Hypoacidity	26.7	9.7	14.8	19.4	16.1
Normal acidity	26.7	61.3	57.4	43.3	51.6
Hyperacidity	6.7	12.9	20.4	26.9	19.4

data of Tables 3 and 4 respectively. We see that the incidence of anacidity in the cases with red blood cell counts under 4 million was 18.2 per cent and 0 per cent in the cases with counts of 5.5 million and over, in the cases with hemoglobin values under 60, the incidence was 40.0 per cent against 11.2 per cent for the cases with hemoglobin values of 80 or over. In general the greater the tendency to achlorhydria, the lower the red blood cell count or hemoglobin content, the greater the tendency to hyperchlorhydria, the higher the red blood cell count or hemoglobin content.

We have also attempted to correlate the gastric acidity with the basal metabolic rate. The results of one or more basal metabolic rates were available in only 41 cases. It is difficult to draw definite conclusions on the basis of this small group. In general, there were large fluctuations in the average free acidity at different levels of metabolism, but there was no discernible trend. The incidence of anacidity seemed to vary to some extent with the level of metabolism as shown in the following tabulation.

Basal metabolic rate <i>per cent</i>	Acidity <i>per cent of cases</i>
Minus 20 to minus 10	0
Minus 9 to minus 1	12.5
0 to plus 9	10.0
Plus 10 and over	42.9

## DISCUSSION

It is always difficult to select individuals who can be considered normal. As previously stated we were careful to exclude any individual who was suffering from any disease which might affect the gastric secretion. However, we included cases with mild gastro-intestinal symptoms when the other findings showed that these symptoms were not significant. Achlorhydria, by itself, was not considered sufficient cause to exclude a case from the normal group. In order to determine whether or not our method of selection caused any errors in the data, we chose a strictly normal group out of the 200 "so-called" normal cases. These patients, 51

in number, had no gastro-intestinal symptoms whatsoever. The data of their gastric analyses were studied as above and found to be similar to those of the larger group. For example the average free acidity was 41.3 cc of 0.1 normal acid and the average total acidity 52.6 cc. The incidence of achlorhydria was 11.8 per cent compared with 13.0 per cent for the larger group.

Therefore it would seem that a patient, considered to have a normal gastric mucosa clinically, and free from gastro-intestinal complaints, may still show a lack of free hydrochloric acid in his gastric secretion. Pollard and Bloomfield (5), however, make the assumption that an organic lesion of the mucosa must exist in these cases, and consequently exclude them from their normal series. There is statistical evidence to show that these cases with anacidity are abnormal because their number is larger than would be expected on the basis of the distribution curve of gastric acidity. Duodenal regurgitation does not account for the large number of cases of anacidity because bile tinged gastric contents were no more frequent in this group than in the group with free acid.

#### CONCLUSIONS

- 1 In a series of gastric analyses of 200 normal patients, using histamine and alcohol as the gastric secretory stimulants, the average free acidity was 40.4 cc of 0.1 normal acid and the average total acidity 50.1 cc. There was no correlation between age and gastric acidity.

- 2 The incidence of anacidity was 13.0 per cent. Up to the age of 65 there was a definite correlation between age and the incidence of anacidity. Beyond this age the number of cases with anacidity dropped off and the number with hyperacidity increased.

- 3 The average gastric acidity was higher in the male than in the female. Consequently females more frequently show anacidity and hypoacidity and less often hyperacidity.

- 4 The red blood cell count and hemoglobin content tended to vary directly with the level of gastric acidity. Consequently the greater the tendency to achlorhydria, the lower the red blood cell count and hemoglobin content, the greater the tendency to hyperchlorhydria, the higher the red blood cell count and hemoglobin content.

- 5 From the data available in 41 cases it seemed that there was some degree of correlation between the incidence of gastric anacidity and level of basal metabolism.

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Secretion in Exophthalmic Goitre and Myxoedema





# THE GASTRIC SECRETION IN EXOPHTHALMIC GOITRE AND MYXOEDEMA

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Our knowledge of the gastric secretion in endocrine disturbances is limited, and this applies to exophthalmic goitre and myxoedema as well as to other endocrine conditions. In both the old and recent literature one finds conflicting statements regarding the gastric contents in thyroid disease. King (1) states that in exophthalmic goitre there may be a complete cessation of secretion of hydrochloric acid resulting in practically an achylia gastrica, and the good results obtained from acid therapy and dietary regulations are sometimes striking. Lockwood (2) studied the gastric contents of 24 patients with hyperthyroidism who had digestive complaints and found 10 or 41.6 per cent showed no free hydrochloric acid, 3 low acid and 11 normal acid. Similarly Moll and Scott (3), in a series of 34 patients with definite exophthalmic goitre, found 19 (56 per cent) with achlorhydria, 10 with hypochlorhydria, 4 with normal acid and 1 with high acid. Herzfeld (4) obtained the following results in 22 cases: achlorhydria and hypoacidity 15, normal acidity 4, and increased acidity 3. Keefer and Bloomfield (5) mention 30 per cent as the incidence of achlorhydria in hyperthyroidism. In a series of 21 cases Moore (6) found achlorhydria in 76 per cent.

On the other hand Nellson (7) considered hyperacidity as one of the early signs of beginning hyperthyroidism. Lewit (8) reported that thyroidin given subcutaneously or by mouth resulted in an increase in the acidity of the gastric secretion in the majority (69 per cent) of 26 patients examined. Badytkes (9), feeding desiccated thyroid, found a depression of the gastric secretion. Boenheim (10), investigating 8 patients, found no characteristic gastric secretion in exophthalmic goitre. Finally Lewit (8), summarizing the contradictory results found in the European literature on this subject, came to the conclusion that the gastric secretion varied with the severity of the disease. In 10 cases of classical Graves' disease he found 5 with no free hydrochloric acid, 4 with low acidity and 1 with normal acidity. In 6 cases of mild hyperthyroidism—"Basedowismus"—hyperacidity was the rule, and in 11 cases of "forme fruste" normal acidity prevailed.

The same confusion exists in the reports on the gastric secretion in myxoedema and hypothyroidism. In 1920, Katz (11) cited cases with low thyroid function and hyperacidity relieved by thyroid. More recently Levy (12) found hyperacidity in all but one of 10 cases of hypothyroidism. In 2 cases which he considered to be myxoedema,<sup>1</sup> thyroid medication depressed the acidity. In agreement with these results is the statement of Hutton (13) that hypothyroidism is associated with a high incidence of peptic ulcer.

On the other hand, Boenheim (10) observed achlorhydria in the 3 patients studied by him. In two of these, gastric acidity became normal after thyroid medication. Sturgis (14) reported the results of the gastric analyses of 5 cases of myxoedema. Three showed no free hydrochloric acid in fasting contents and a low total acidity following the test meal, one showed normal acid, and one low acid in both fasting and test meal samples. Lockwood (2) also reported on 10 cases of myxoedema and found achlorhydria in 60 per cent.

The experimental data in this subject are also contradictory. In 1916 Hardt (15) demonstrated that in dogs with Pawlow pouches large doses of thyroid by mouth depressed the gastric secretion. Truesdell (16), working in the same laboratory, confirmed this result. A year later, Chang and Sloan (17) showed that this is true also when dogs are fed small doses of thyroid. After thyroidectomy they found a marked rise in the volume of the gastric secretion as well as a rise in gastric acidity.

On the other hand, Rogers, Rahe and Ablahadian (18) showed that alkaline saline solutions or alcohol extracts of thyroid administered subcutaneously caused a vigorous stimulation of the gastric secretion. Lewit (8) confirmed this partly in that he found that intravenous thyroïdin in dogs resulted in an increase in the volume of gastric secretion, but no change in the acidity. He also cited the work of Ponirowski to the effect that thyroidectomy diminishes the secretion of gastric juice and thyroid subcutaneously increases it.

A general criticism may be applied to all the results obtained by the above investigators, particularly those who dealt with the incidence of achlorhydria in thyroid disease, namely, that the gastric juice was obtained by means of test meals and not histamine. It is now well recognized that true achlorhydria cannot be diagnosed with any certainty unless the gastric juice is obtained by means of a maximum stimulation as with histamine. We have been unable to find any reports in the literature on the gastric contents in patients with thyroid disease obtained by this means. This study was, therefore, carried out in an attempt to obtain

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<sup>1</sup> The following metabolic data given in the text throw doubt on this diagnosis. Case 8 Basal metabolic rate -20 before treatment, -25 after treatment. Case 9 Basal metabolic rate -20 before treatment, and -16 after treatment.

definite knowledge of the gastric secretion in exophthalmic goitre and in myxoedema, using histamine as the main stimulus for the secretion of gastric juice

#### METHOD

The technique of obtaining the gastric contents is described in another paper dealing with the gastric analysis in normal people (19). Briefly, it consists in the analysis of three samples of gastric contents, i.e., the fasting contents, and the samples obtained one half hour and one hour after the subcutaneous injection of 0.5 mgm. ergamine acid phosphate (histamine) and the ingestion of 50 cc. of a 7 per cent alcoholic solution. The volume, the free and total acidity of each sample were recorded, using Töpfer's reagent and phenolphthalein as the indicators in the titration of the respective acidities. The results were compared with normals and correlated with various factors. The method of obtaining the normal data is described in the paper referred to above. The analyses were done by various members of the resident medical staff.

#### MATERIAL

##### *Exophthalmic goitre*

A total of 50 patients with exophthalmic goitre had gastric analyses done by the method described. Almost all the patients were in the age groups of 20-59, only one below 20 and one over 59 years of age. The majority of patients were under 40 years of age. There were 18 males and 32 females. The results for free and total acidity in relation to age of patients are tabulated in Table I. This, as well as the subsequent tables, is based on the maximum acidity of the gastric juice following stimulation with histamine and alcohol.

The striking result is the high incidence of anacidity—38 per cent for the group. The incidence of anacidity in 200 normal patients (19) examined the same way was found to be 13 per cent. The result becomes all the more significant if the comparison is made with those patients falling into the same age groups as our exophthalmic goitre patients, and a correction applied for the difference in age distribution. This has been done in Figure 1. It shows that the corrected incidence of anacidity in exophthalmic goitre is 45.6 per cent compared to the normal of 12.6 per cent. This chart also shows that hypacidity is higher in the exophthalmic goitre group than in the normal, whereas hyperacidity is much lower. The results for total acidity run more or less parallel with those for free acidity. The correction for sex distribution as compared to the normals is omitted because it does not alter the results significantly.

The relatively low acidity of the gastric secretion in exophthalmic goitre patients can be demonstrated in another way by comparing their average acidities with those of the normals according to age groups. This is done graphically in Figures 2 and 3. In general, normal people secrete

almost twice as much acid in the stomach as patients with exophthalmic goitre, the actual values being 40.4 cc and 21.0 cc respectively

It has been shown by Bloomfield and Keefer (20) that physical fitness affects the degree of gastric acidity. We divided our patients on the basis of their general physical condition into good, fair and poor—at best an approximation. There was apparently no correlation between physical fitness and gastric acidity in these cases. The average free acidity of the 29 patients in good condition was 20.7 cc N/10 per 100 cc and of the

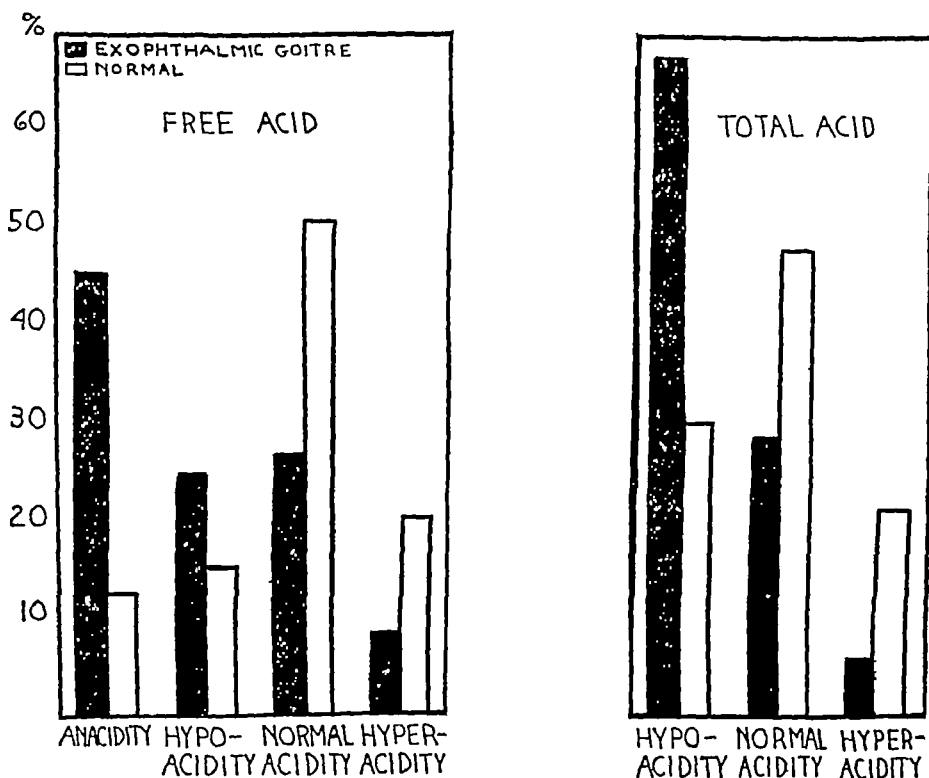


FIG 1 THE PERCENTAGE INCIDENCE OF THE VARIOUS DEGREES OF ACIDITY OF 159 NORMAL PATIENTS UNDER 60 YEARS OF AGE AND OF 50 PATIENTS WITH EXOPHTHALMIC GOITRE, CORRECTED FOR DIFFERENCES IN AGE DISTRIBUTION

19 patients in fair condition 23.1 cc N/10 per 100 cc. The two patients found to be in poor condition may be disregarded. Since the probable errors of these averages are between two and three, it is obvious that these averages do not differ by any significant amount.

Bloomfield and Keefer (20), as well as other writers, stress the relationship between age and degree of gastric acidity. An analysis of Table I and Figures 2 and 3 shows that in exophthalmic goitre, at least, this is not definite. For example, the coefficient of correlation between age and free acidity is  $-0.21 \pm 0.091$ , a figure which must be interpreted with

caution The apparent slight correlation is entirely due to the cases without free hydrochloric acid The cases possessing any amount of free acidity show no correlation with age

We have also analyzed our data with respect to the relationship of acidity to the level of the red blood cell count, the hemoglobin, and the basal metabolic rate before iodine medication The results here also indicate that these factors are not definitely related to the degree of gastric acidity, except in the case of achlorhydria to be discussed below For example, of the 12 patients with hypochlorhydria (free acid) none had

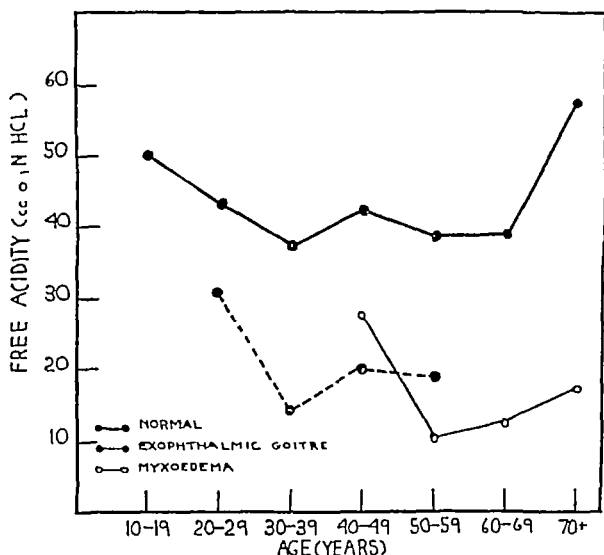


FIG 2 THE AVERAGE FREE ACIDITY BY AGE GROUPS OF 200 NORMAL PATIENTS, 50 WITH EXOPHTHALMIC GOITRE AND 17 WITH MYXOEDEMA

a red count below 4.5 million and only one had a hemoglobin below 70 per cent. Of the 15 with normal acidity, 3 had a red count below 4.5 million and none a hemoglobin below 70 per cent. The corresponding figures for the 4 patients with hyperchlorhydria were one and two respectively

Another way of expressing the relationship is to calculate the coefficients of correlation Between the acidity and the red blood cell count the values are  $+0.0002 \pm 0.095$  for free acid and  $+0.018 \pm 0.100$  for total acid, between the acidity and the hemoglobin they are  $+0.29 \pm 0.087$  and  $+0.22 \pm 0.091$  for free and total acid, respectively The

former show no degree of correlation whatever, the latter show a slight degree which, however, disappears when the achlorhydria cases are separated from the main group

The data for the relationship between the acidity of the gastric juice and the height of the metabolism are given in Table II. The coefficients of correlation are  $-0.27 \pm 0.088$  for free acidity and  $-0.29 \pm 0.087$  for total acidity. As a result of the scattered distribution, these figures are not highly significant. Nevertheless, they indicate a probable inverse relationship between the level of metabolism and gastric acidity. As

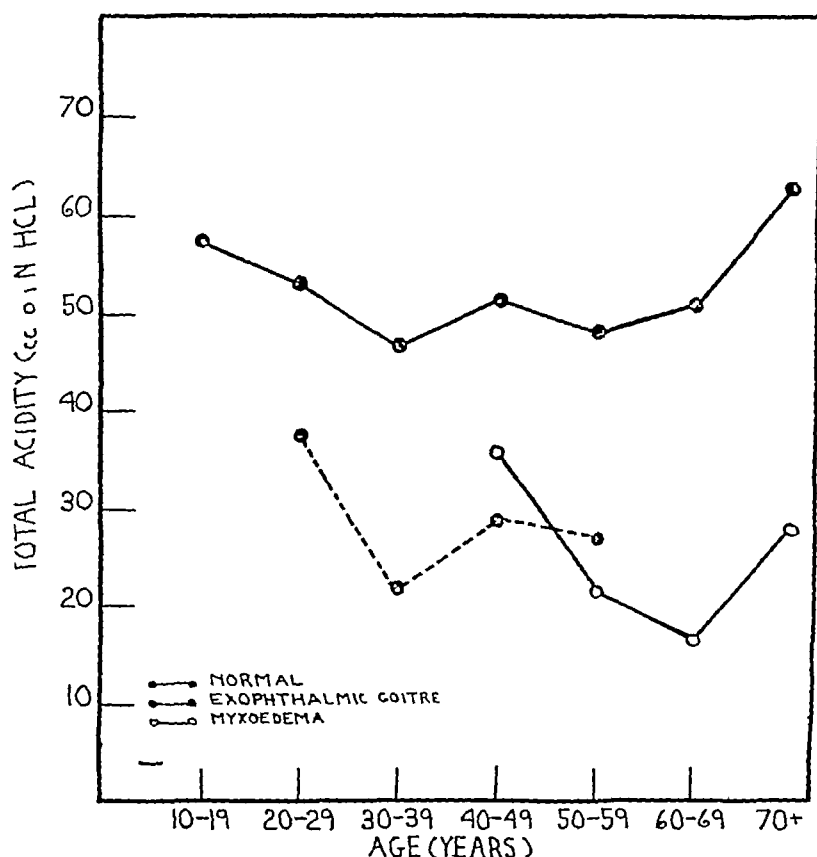


FIG 3 THE AVERAGE TOTAL ACIDITY BY AGE GROUPS OF 200 NORMAL PATIENTS, 50 WITH EXOPHTHALMIC GOITRE AND 16 WITH MYXOEDEMA

judged from the averages of Table II, this relationship is not a progressive one. There is a sudden change at the 50-59 interval of metabolism, but not much variation before and after it. Again, if the cases with anacidity are separated out, it is found that the remainder show no correlation between acidity and level of metabolism, and all the averages have similar values. Thus, the coefficient for the 31 cases with free acid is  $-0.08 \pm 0.122$ , which is smaller than its probable error.

TABLE I

*Distribution by age of the free and total acidity of 50 patients with exophthalmic goitre*

Cc. 0.1 N HCl	Free acidity					Total acidity				
	Age (years)				Total	Age (years)				Total
	-29	30-39	40-49	50+		-29	30-39	40-49	50+	
<i>per 100 cc.</i>	<i>cases</i>	<i>cases</i>	<i>cases</i>	<i>cases</i>	<i>cases</i>	<i>cases</i>	<i>cases</i>	<i>cases</i>	<i>cases</i>	<i>cases</i>
0	2	9	5	3	19			1		1
1-9	1	2		2	5	3	8	3	2	16
10-19	4	2	1		7	1	3		3	7
20-29	1	1			2	3	2	2		7
30-39	1	1	2	1	5	1	2		1	4
40-49	2	1	1		4	2	1	1		4
50-59	2				2	2		1		3
60-69		2			2	1		1		2
70-79	2		1		3	1	1	1		3
80+				1	1	1	1		1	3
Total	15	18	10	7	50	15	18	10	7	50
Averages	30.7	14.2	20.3	19.0		37.5	21.7	28.9	26.9	
Standard deviation of acidity = 24.1						Standard deviation of acidity = 26.6				
Standard deviation of age = 10.5						Standard deviation of age = 10.5				

TABLE II

*Distribution according to level of metabolism of the free and total acidity of 50 patients with exophthalmic goitre*

Cc. 0.1 N HCl	Basal metabolic rate (per cent)													
	Free acidity							Total acidity						
	-30	30-39	40-49	50-59	60-69	70+	Total	-30	30-39	40-49	50-59	60-69	70+	Total
<i>per 100 cc.</i>	<i>cases</i>	<i>cases</i>	<i>cases</i>	<i>cases</i>	<i>cases</i>	<i>cases</i>	<i>cases</i>	<i>cases</i>	<i>cases</i>	<i>cases</i>	<i>cases</i>	<i>cases</i>	<i>cases</i>	<i>cases</i>
0	1	3	5	3	3	4	19						1	1
1-9	1	1	3	1			5	2	3	5	4	2	3	16
10-19	1	2	3			1	7	1	3	3				7
20-29	1		1				2	1	1	3		1	1	7
30-39	1	1	1	1	1		5	1		2		1		4
40-49	2	2				1	4	1		2				4
50-59		1					2	2			1			3
60-69	1		1				2	1					1	2
70-79		1		2			3	1	1	1				3
80+.			1				1			3				3
Total	7	11	17	5	4	6	50	7	11	17	5	4	6	50
Averages	32.4	25.5	23.9	8.2	8.3	10.5		40.7	33.3	31.8	15.8	16.5	15.5	
Averages 31 cases with free acid	37.8	35.0	33.9	27.4										

Standard deviation of acidity = 24.4  
 Standard deviation of basal metabolic rate = 15.1

Standard deviation of acidity = 26.6  
 Standard deviation of basal metabolic rate = 15.1



It is interesting to note that the gastric secretion in exophthalmic goitre varies to some extent in the sexes. The results for free acidity are as follows

	Males (18) <i>per cent</i>	Females (32) <i>per cent</i>
Anacidity	50.0	31.3
Hypoacidity	11.1	31.3
Normal acidity	22.2	34.3
Hyperacidity	16.7	3.1

It is obvious that males have more of achlorhydria but also of hyperchlorhydria. That these results are significant and not due to chance may be verified to some degree by application of the chi-square test. Thus it is found that the odds are 10 to 1 against the occurrence of such results on the basis of chance alone. These differences in the sexes are unlike those found by us for normal people. The latter show a slightly increased incidence of hypoacidity among females and of hyperacidity among males. The differences existing in the exophthalmic goitre patients may be explained partly by the inequality in the severity of the disease. The average basal metabolic rate of the male patients was plus 52.0 per cent and of the female patients plus 42.4 per cent. Since the incidence of achlorhydria, as shown below, probably varies with the level of metabolism, then it is to be expected that males should have a higher incidence of achlorhydria. The higher incidence of hyperchlorhydria, however, appears to be paradoxical.

#### *Achlorhydria*

As indicated previously, of the 50 patients with exophthalmic goitre studied, 19 or 38 per cent showed achlorhydria after stimulation with histamine. This percentage becomes all the more significant when compared with the expected result based on normals. The assumption of a general depression of acidity in exophthalmic goitre will not entirely explain this high value. Consequently, it is likely that other factors play a rôle in the causation of achlorhydria.

Unlike the group which secretes free acid, the achlorhydria group shows definite correlations with some of the factors studied. Age, red blood cell count level, hemoglobin content and basal metabolic rate are more or less definitely related to the incidence of anacidity. These relationships are shown graphically in Figures 4a and 4b. We see that 8 of the 19 patients showing achlorhydria were 40 years of age or more. The incidence of achlorhydria for this age group was 47 per cent, whereas for those under 40 years it was 33 per cent. Of the 16 patients with red blood cell counts under 4.75 million, achlorhydria was present in 56 per cent, whereas of the 34 patients with counts of 4.75 million and over it was present in 29 per cent. The percentages for the 25 patients with hemoglobin values under 75 and the 25 patients with values of 75 per cent and over were 48 and 28 respectively. Similarly, 67 per cent of the 15

patients with metabolisms of plus 50 or more showed achlorhydria and only 26 per cent of the remainder under plus 50 showed this

The above results must be interpreted with caution Their significance can be determined to some extent by means of their correlation

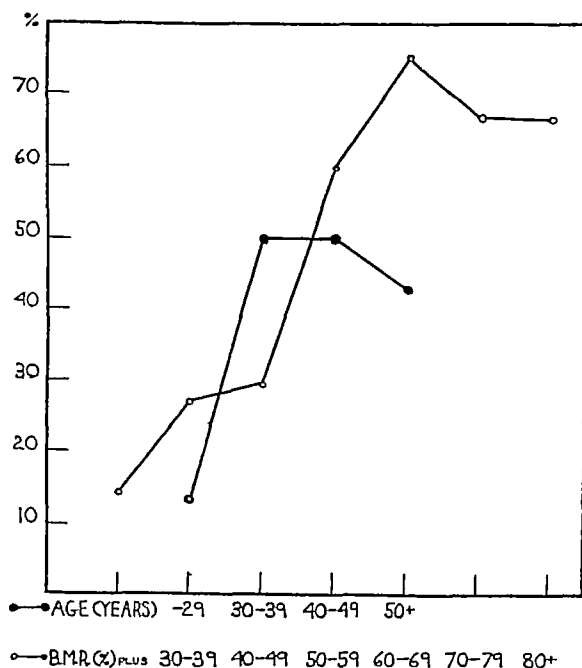


FIG 4a THE PERCENTAGE INCIDENCE OF ACHLORHYDRIA IN EXOPHTHALMIC GOITRE IN RELATION TO AGE AND BASAL METABOLISM

coefficients This has been done by the Rank-Difference Method and the results listed in Table III They indicate that there are high degrees of

TABLE III

*The correlation between the incidence of achlorhydria in exophthalmic goitre and the factors of age red blood cell count hemoglobin and level of basal metabolic rate*

Correlation of achlorhydria with	Coefficient of correlation	Probable error	Coefficient Probable error
Age	+0.69	$\pm 0.125$	5.5
Red blood cell count	-0.56	$\pm 0.198$	2.8
Hemoglobin	-0.54	$\pm 0.158$	3.4
Basal metabolic rate	+0.93	$\pm 0.029$	32.1

correlation between the incidence of achlorhydria and age level, and achlorhydria and the level of metabolism. On the other hand, there are only probable correlations (inverse) for red blood cell count and hemoglobin, of slightly greater significance than those of the entire group. The factors of age and height of metabolism are probably independent as far as their influence on the gastric secretion is concerned because most of the patients with high metabolic rates were young.

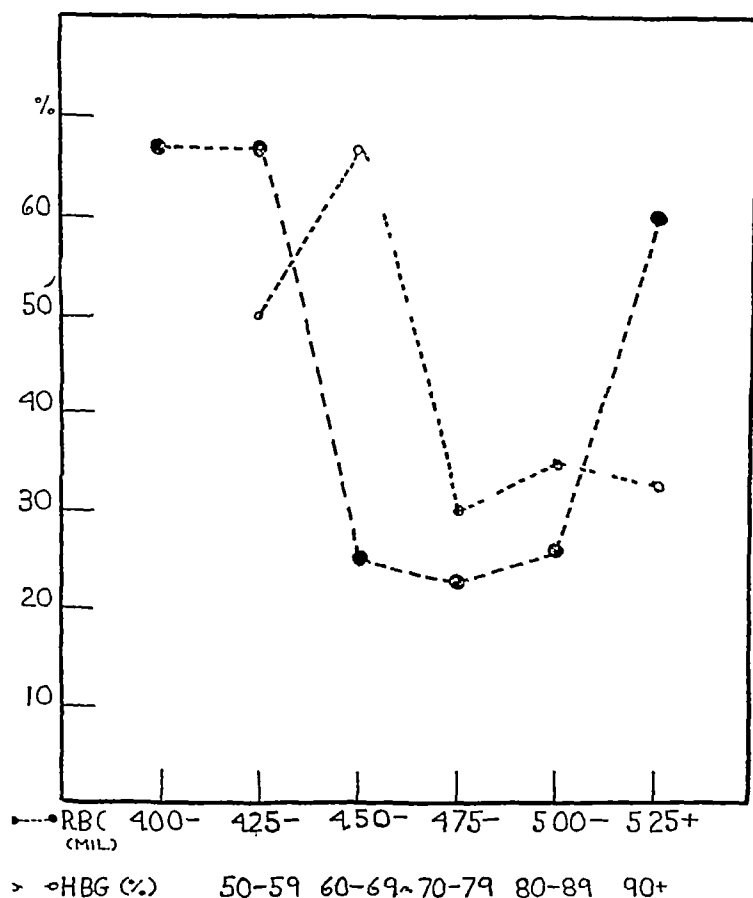


FIG 4b THE PERCENTAGE INCIDENCE OF ACHLORHYDRIA IN RELATION TO RED BLOOD CELL COUNT AND HEMOGLOBIN

### *Myxoedema*

Seventeen patients with myxoedema were studied in the hospital. The gastric contents in all cases were examined by the same method as was used in the normal and exophthalmic goitre groups. All but two of the patients were females. The ages ranged from 36 to 73. The results by age are recorded in Table IV. The data for total acidity were lacking in one case.

Here also the striking results are the relatively low acidity in the group as a whole (Figures 2 and 3) and the high incidence of achlorhydria. As in the case of exophthalmic goitre, the results become all the more significant when compared with a normal series after correction for age distribution (Figure 5). Nine of the 17 patients, or 53 per cent, showed achlorhydria. Corrected for age distribution the figure becomes 75 per cent compared to 15 per cent incidence in 132 normals of the same age

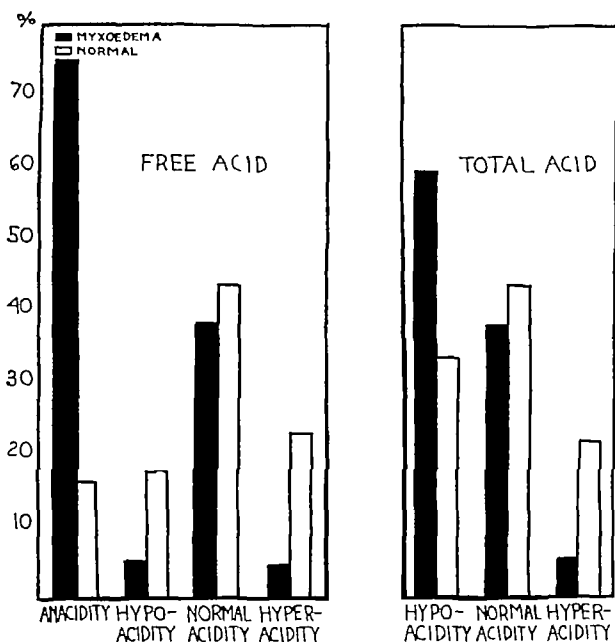


FIG 5 THE PERCENTAGE INCIDENCE OF THE VARIOUS DEGREES OF ACIDITY OF 132 NORMAL PATIENTS AGED 40 AND OVER AND 17 PATIENTS (16 FOR TOTAL ACID) WITH MYXOEDEMA CORRECTED FOR DIFFERENCES IN AGE DISTRIBUTION

groups. Similarly, the corrected incidence of hyperchlorhydria (free acid) is 4.8 per cent against the normal of 23.5 per cent. The other degrees of acidity are also lower than normal, but not to the same extent. The correction for sex distribution makes the result for the incidence of achlorhydria 67 per cent, but does not alter the other figures significantly.

This brings up the same problem as already discussed in dealing with exophthalmic goitre, namely what factors, besides general depression of

gastric secretion, operate in these cases to cause achlorhydria? That such factors do exist must be inferred from the high incidence of achlorhydria

The acidity of myxoedema has also been correlated with various factors such as age (Table IV), red blood cell count, hemoglobin, and

TABLE IV  
*Distribution by age of the free and total acidity in patients with myxoedema*

Cc 0.1 N HCl  per 100 cc	Free acidity					Total acidity				
	Age (years)				Total	Age (years)				Total
	-49	50-59	60-69	70+		-49	50-59	60-69	70+	
	<i>cases</i>	<i>cases</i>	<i>cases</i>	<i>cases</i>	<i>cases</i>	<i>cases</i>	<i>cases</i>	<i>cases</i>	<i>cases</i>	<i>cases</i>
0	4	2	1	2	9		1			1
1-9	1				1	3		1	1	5
10-19						2			1	3
20-29			1		1					
30-39	1	1			2			1		1
40-49						1	1			2
50-59				1	1					
60-69	2				2	1			1	2
70-79						1				1
80+	1				1	1				1
Total	9	3	2	3	17	9	2*	2	3	16
Averages	27.6	10.3	12.5	17.3		35.9	21.5	16.5	27.7	
Standard deviation of age = 10.8						Standard deviation of age = 10.8				
Standard deviation of free acidity = 27.8						Standard deviation of total acidity = 29.1				

\* One patient in this group was found to have no free acid following histamine, but total acidity was not recorded

basal metabolic rate There is obviously no correlation between age and incidence of achlorhydria In the case of red blood cells and hemoglobin, the findings are a little different Nine of the 17 patients had counts below 4 million red blood cells and three below 3 million The average free acidity of the 9 patients with counts under 4 million was 20.2 cc of 0.1 N hydrochloric acid, and of the 8 patients with counts over 4 million it was 21.8 cc, values which indicate that the red blood cell level on the whole is not related to acidity On the other hand, of the 9 patients with achlorhydria, 6 had counts below 4 million and 2 below 2 million cells Of the 8 cases showing free acid, only 3 had counts below 4 million Also, the incidence of achlorhydria in the cases with counts under 4 million cells was 67 per cent against 38 per cent in the cases with counts of 4

million cells and over. It seems, therefore, that low red blood cell counts are more frequent among patients with achlorhydria than among those with free acid. In the case of hemoglobin, the average acidity is more closely related to the level of hemoglobin. Twelve cases with hemoglobin values under 75 per cent averaged 14.8 cc of 0.1 N free acid, whereas the remaining five averaged 35.8 cc. High values of acidity occurred less often with low hemoglobin figures than with high ones. Eight of the 9 cases with achlorhydria had hemoglobin values below 75 per cent. Of the 12 cases with hemoglobin values under 75 per cent, 8 had no free acid, whereas only one of the 5 remaining cases had an acidity.

The effect of metabolism on the acidity of the gastric juice is also definite but slight. The average free acidity of 11 patients with metabolic rates greater than minus 30 was 13.1 cc of 0.1 N acid and of the 6 remaining patients was 35.3 cc. Similarly, the 3 patients in the group minus 10 to minus 19 all had achlorhydria, whereas the three highest acidities occurred in the group of minus 30 to minus 39. These results are contrary to what might be expected. Since there is a general depression in the gastric secretion in myxoedema, it is only natural to expect that the more severe the disease, the greater the depression. Here also is evidence that factors other than depression alone play a rôle in producing the changes in the gastric secretion.

These relationships may be further clarified by treating the data mathematically. In Table V are listed the coefficients of correlation

TABLE V

*The correlation between acidity in myxoedema and age, red blood cell count, hemoglobin and basal metabolic rate*

Correlation of acidity with	Free acidity			Total acidity		
	Coefficient of correlation	Probable error	Coefficient Probable error	Coefficient of correlation	Probable error	Coefficient Probable error
Age	-0.16	±0.167	1.0	-0.17	±0.171	1.0
Red blood cell count	+0.26	±0.160	1.6	+0.077	±0.176	0.4
Hemoglobin	+0.36	±0.149	2.4	+0.22	±0.168	1.3
Basal metabolic rate	+0.27	±0.159	1.7	+0.35	±0.155	2.3

between acidity and the above mentioned factors. These figures are more or less confirmatory in that they indicate probable slight correlations but no certain correlations. It must be remembered that results based on 17 cases are to be interpreted with caution. Definite conclusions regarding the relationship between gastric acidity and age, red blood cell count, hemoglobin and basal metabolic rate cannot be drawn until many more cases of myxoedema have been studied.

## DISCUSSION

The lowering of the acidity of the gastric secretion in exophthalmic goitre and in myxoedema is definite and clearcut. On the basis of experimental findings quoted previously, these results are certainly unexpected. If the activity of the thyroid alone played the major rôle, then there should be found hypoacidity in exophthalmic goitre and hyperacidity in myxoedema. Consequently, we must infer that probably other factors also play a rôle in affecting the gastric secretion in these conditions.

As already mentioned, the data indicate that depression alone of a previously normal acidity will not account for the high incidence of achlorhydria in these two diseases. In general, the average acidity is about half the normal value, whereas the incidence of achlorhydria is fourfold and fivefold that of normal in exophthalmic goitre and myxoedema respectively. The possibility of a threshold phenomenon must be considered, i.e., depression of the gastric secretion occurs up to a point beyond which achlorhydria will result. This threshold, if such there be, must vary with different people. Lewit (8) suggests an explanation of achlorhydria in exophthalmic goitre on an anatomical basis. The gastric mucosa is functionally incompetent as a result of diffuse lymphocytic infiltration. Consequently, it is very susceptible to traumatic influences which may lead to atrophy. One might attempt to explain the achlorhydria on the basis of regurgitation of alkaline duodenal contents into the stomach. If this were true, then the total acidities would be relatively high, and bile-tinged gastric contents would be frequent. Our analyses, however, showed that the total acidities in the cases of achlorhydria were low. Bile-tinged secretion was mentioned in 8 cases of exophthalmic goitre and in 2 cases of myxoedema. Still another explanation is that the achlorhydria and these two diseases have predisposing constitutional factors in common.

The question naturally arises whether the achlorhydria is permanent or not. If constitutional, the achlorhydria would be expected to persist. If acquired as a result of the disease, then it still might be permanent because of damage to the acid secretory mechanism of the stomach. The findings of Lewit (8) that 10 of 17 autopsied patients showed gross atrophy of the gastric mucosa would support this idea if it were not for the fact that postmortem changes make such observations on the stomach extremely unreliable.

Further investigation of this problem is necessary. We have at present only indirect evidence on this point. Although most authors feel that the combination of exophthalmic goitre and pernicious anemia in the same person is extremely rare, Meulengracht (21) found 8 cases out of a total of 151 pernicious anemia patients, associated with exophthalmic goitre. Most of them developed the anemia subsequent to the thyroid disease and achlorhydria was known to be present for several years before





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# EFFECTS OF ENVIRONMENTAL TEMPERATURE, ANESTHESIA AND LUMBAR SYMPATHETIC GANGLIONECTOMY ON THE TEMPERATURES OF THE EXTREMITIES OF ANIMALS<sup>1</sup>

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## INTRODUCTION

The reports of alleviation of symptoms following sympathetic ganglionectomy and trunk resection in certain cases of peripheral vascular diseases, arthritis and scleroderma have created a noticeable interest in the underlying physiology of the sympathetic nervous system when functioning in a normal or abnormal fashion. From clinical as well as experimental data the changes which take place following sympathetic ganglionectomy with trunk resection have proved to be chiefly vasomotor. The relief of pain in certain cases may be brought about by this same mechanism or may be the result of interrupted sensory pathways. The postoperative vasomotor changes consist mainly of lessened vasoconstriction. This can be demonstrated in the extremities of animals as well as of patients with normal blood vessels through the use of physical apparatus for measuring the changes in superficial temperatures produced by operation. Believing that this vasomotor action is present in vascular diseases of the peripheral vessels in varying amounts dependent on the interplay of environmental conditions and nervous mechanism, Morton and Scott (1) called attention to a definite temperature gradient beginning proximally and increasing distally (vasoconstrictor gradient) in persons with normal peripheral vessels and that, 'after spinal anesthesia, there is usually a rapid rise in surface temperature on the feet so that all surface areas of the body reach approximately the same level (normal vasodilatation level). Superficial temperatures also showed rapid changes in normal persons under inhalation anesthesia. In vascular diseases, therefore, the use of spinal anesthesia produces a selective effect on the vasoconstrictors and the degree of vasocontraction may be measured by the

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<sup>1</sup> Read before the Section on Pathology and Physiology at the Eighty second Annual Session of the American Medical Association Philadelphia Pa., June 8 to 12 1931.

changes in the superficial temperatures of the extremities White (2) also recently advocated blocking the sympathetic nerves to the extremities to determine the presence or absence of vasoconstriction

#### PURPOSE OF THE INVESTIGATIONS

In addition to observing the reactions of the vasomotor mechanism as shown by changes of superficial, subcutaneous and interdigital temperatures of the extremities of animals (dogs) under various types of anesthesia in a fairly constant environment ( $24^{\circ}$  to  $28^{\circ}$  C), we were interested in producing a rapidly changing environment and in making observations on the vasomotor reactions obtained in the same dogs in normal and anesthetized states when under similar constant or rapidly changing environmental conditions After determining the reactions of the normal vasomotor activities with and without anesthesia to a rapidly changing environment, similar procedures were carried out following sympathetic denervation of one hind extremity in order to determine whether there was a marked difference in vasoconstriction in the two extremities demonstrated peripherally or in the tissues by measurements of superficial, subcutaneous and intermetatarsal temperatures with thermocouples

#### EXPERIMENTAL METHODS AND SURGICAL PROCEDURES

*Measurements of temperature by thermocouples* Superficial, subcutaneous and intermetatarsal temperatures were measured by means of the electromotive thermometer devised and described by Sheard (3) This instrument is equipped with numerous thermocouples, each of which is inserted in a needle with the thermojunction near the tip or on the surface of a fiber or hard rubber button Diagrammatic sketches of the electromotive thermometer are shown in Figure 1 A thermojunction, connected to all the thermojunctions which may be used for obtaining measurements of temperature, is inserted in a thermostat maintained electrically at a given, constant temperature The readings of the galvanometer included in the thermocouple circuits are converted into equivalent thermal readings Having calibrated the deflections of the galvanometer in equivalent thermal readings, any of the various thermocouples with which the apparatus is equipped may be applied to or inserted into the body and the temperatures read directly from the calibrated scale By means of this ensemble it is possible to read the temperature indicated by any of the thermocouples applied to or inserted in the body in about ten seconds of time Figure 2 is a photograph of the instrument used in these investigations Each switch is connected to the common thermojunction and to a specified thermocouple Some of the forms of thermojunctions for the measurement of superficial, subcutaneous and intermetatarsal temperatures are shown

*Situation of the thermocouples* In general a thermocouple was applied to or inserted in each of the two hind extremities as follows (1)

subcutaneously, on the inner side of the leg and above the patella, (2) superficially, between the toes, and (3) intermetatarsally, that is, in an interosseous position but relatively near the surface of the skin of the foot

In the presentation of our experimental data we shall refer to the situation of the various thermocouples as (1) left interdigital, (2) right interdigital, (3) left subcutaneous, (4) right subcutaneous, (5) environmental temperature of hind extremities, (6) right intermetatarsal and (7) left intermetatarsal

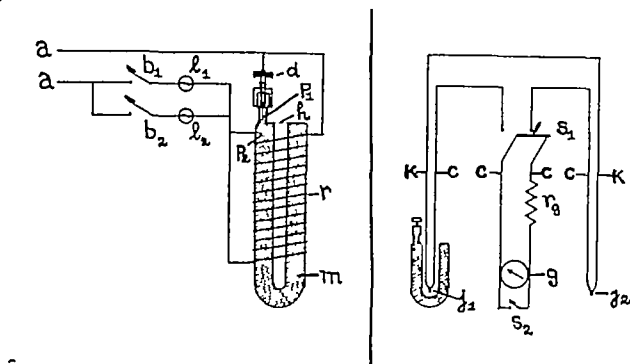


FIG 1 ELECTROMOTIVE THERMOMETER

Left hand diagram shows the thermostat and heating circuit  $a-a$ , 110 volt circuit  $b_1$  and  $b_2$  switches for inserting auxiliary resistances (incandescent lamp bulbs)  $l_1$  and  $l_2$  in the circuit  $d$  adjustable screw to set the temperature of the thermostat  $p_1$  and  $p$  platinum wires which with mercury form the thermojunction  $h$  inner tube of thermostat which carries one set of thermojunctions or one thermojunction common to all junctions  $r$ , resistance for heating the thermostat  $m$ , mercury right hand diagram shows the circuit containing the thermocouple and the galvanometer  $j_1$  and  $j_2$ , thermojunctions of copper,  $c$  and constantan  $k$   $r_g$  auxiliary resistance in galvanometric system  $g$  galvanometer  $s_1$  switch for including the galvanometer in the thermoelectric circuit  $s_2$ , shunt to damp the galvanometer quickly

*Method of rapidly changing the environmental temperature* In general the temperature of the room in which the various operations and measurements of temperature were made ranged from  $23^\circ$  to  $27^\circ$  C. The initial portions of the curves numbered 5 in the various portions of Figures 4 and 5 record the values of the environmental temperatures during the ten to twenty minutes of the different experiments. After the data had been obtained on the values of the superficial, subcutaneous and intermetatarsal temperatures under the conditions of the temperature of the room, the environmental temperatures of the lower extremities were reduced rapidly from room temperature to approximately  $0^\circ$  C. The lower extremities

were housed in a chamber made of two parts. The lower portion of this cooling chamber is an extension of, or addition to, the frame to which the dog was attached by means of gauze taping. The extremities rested on a metallic netting which occupied the width of the frame. Underneath the sheet of wire mesh was placed a small cylinder of carbon dioxide which was used for lowering the temperature of the chamber. The neck of the cylinder fitted into and through a suitable opening in the side of the frame and in such fashion that the flow of gas from the cylinder into the chamber could be controlled from the outside. After the insertion or attachment of the various thermocouples, the cover was placed in position and the opening in the end which fitted over the body of the dog was blanketed. A mercury thermometer inserted through the top of the chamber registered the temperature within the chamber.

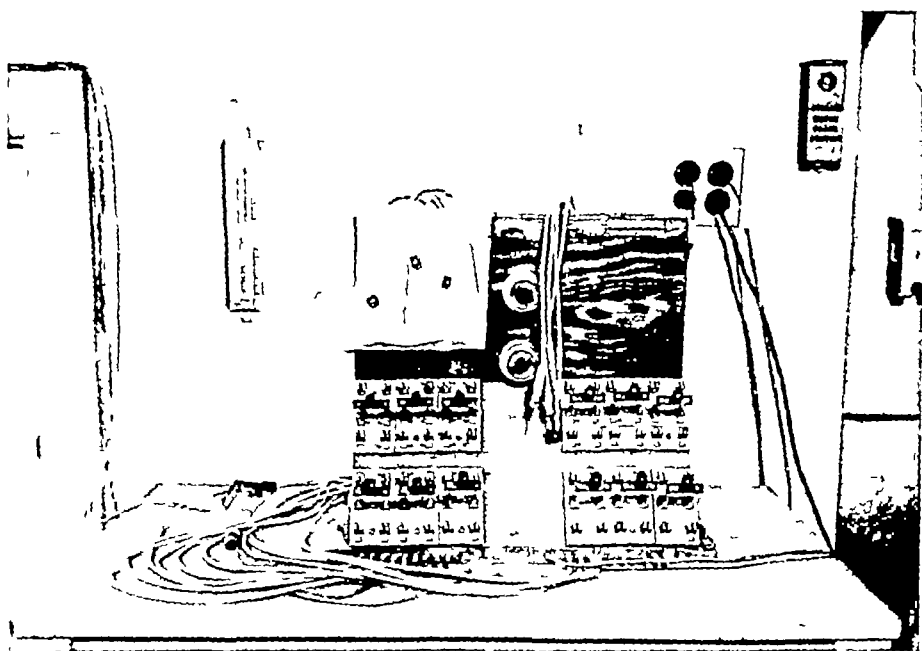


FIG. 2 ELECTROMOTIVE THERMOMETER, TYPES OF THERMOJUNCTIONS AND SWITCHES CONNECTED TO EACH OF THE THERMOCOUPLES, RESPECTIVELY

Figure 3 is a photograph of a cylinder (size *D*) of carbon dioxide to which is attached, by means of a yoke, a metallic tube which is closed at its distal end and which is punctured with a number of fine holes. When the tank is opened, the gas at fairly high pressure escapes through the numerous small openings in the metallic pipe. By reason of the rapid expansion of the gas as it issues into the air at atmospheric pressure the temperature of the surrounding air is reduced rapidly. The temperature of the chamber could be reduced from  $25^{\circ}\text{C}$  to the neighborhood of the freezing point in from one minute to two minutes. The curves

marked 5 in the various portions of Figures 4 to 6 inclusive show the course of the relationship between the time in minutes and the environmental temperature

*Surgical procedures and data* Five dogs were used in these experiments. After determining the reactions of the normal vasomotor activity, as evidenced by measurements of temperature, to a changing environment and with and without anesthesia, similar sets of observations were made following sympathetic denervation of one of the hind extremities. The experimental data obtained under ether administered by inhalation, amytal intravenously, and procaine intraspinally were considered basic. In four of the animals the lumbar sympathetic ganglia were removed on the left side and in the fifth dog on the right side.

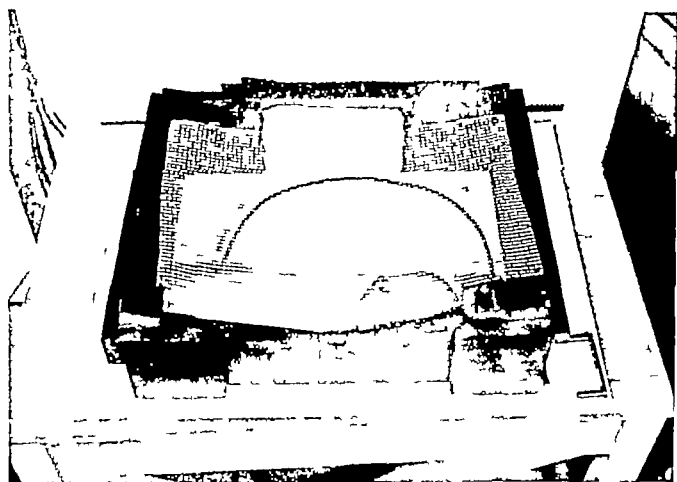


FIG. 3. LOWER HALF OF THE COOLING CHAMBER. THE TANK OF CARBON DIOXIDE AND METALLIC TUBE IN POSITION ARE SHOWN.

Experiment 1. October 9, 1930. Under general anesthesia a left abdominal incision was made through the skin and muscles and the peritoneal cavity was opened. After retraction of the viscera and incision of the posterior peritoneum, the lumbar sympathetic chain was exposed and removed.

Experiment 2. November 25, 1930, left sympathetic ganglionectomy and trunk resection were performed.

Experiment 3. January 7, 1931, left sympathetic ganglionectomy and trunk resection were performed.

Experiment 4 February 6, 1931, under general anesthesia, a right abdominal incision was made through the skin and muscle and the peritoneal cavity was opened. After retraction of the viscera and abdominal vessels, the right lumbar sympathetic ganglia were removed.

Experiment 5 February 25, 1931, left lumbar sympathetic ganglionectomy and trunk resection were performed.

#### EXPERIMENTAL DATA AND RESULTS

Figures 4 and 5 present in graphic form the data obtained through the measurements of temperature by thermocouples in experiment 4 and in experiment 5 (Fig 5). In all portions of both figures the curves numbered 5 show the course of the changes in environmental temperature. Curves 1, 3 and 7 refer to the time-temperature relationships obtained interdigitally on the left foot, subcutaneously in the region of the patella and intermetatarsally in the left foot, respectively. Curves 2, 4 and 6 have similar significance with reference to the right hind extremity.

Figures 4 (I) and 5 (I) show the course of the time-temperature relationship and the environmental temperature without anesthesia. Figures 4 (II) and 5 (II), under ether anesthesia, Figures 4 (III) and 5 (III), under ether anesthesia after lumbar sympathetic ganglionectomy (operation performed on the right side in Fig 4 and on the left in Fig 5), and Figures 4 (IV) and 5 (IV), after lumbar sympathetic ganglionectomy and without anesthesia.

The curves of Figures 4 and 5 indicate the following salient points:

- 1 In the normal animal, there was marked vasoconstriction evidenced in the interdigital and intermetatarsal portions of the hind feet when the environmental temperature was rapidly lowered from room temperature (about  $25^{\circ}\text{C}$ ) approximately to the freezing point. This vasoconstriction, as indicated by lowered thermal readings on the thermocouples (curves 1 and 2), persisted for from twenty to sixty minutes, lagged behind the readings of the environmental temperature (which returned fairly rapidly to that of the room as shown in curve 5), and finally recovered to a state which was normal or slightly above normal.

- 2 In the normal animal, the time-temperature relationship of the subcutaneous areas near the patella (curves 3 and 4) paralleled the course of the environmental temperature.

- 3 In the normal dog under ether anesthesia, all subcutaneous and superficial time-temperature curves (curves 1, 2, 3 and 4) showed that there was no evidence of vasoconstriction. In general, the recovery to normal temperature was more rapid than the course of the environmental temperature (curve 5). Also, the drop in subcutaneous and superficial temperatures under ether anesthesia was less than in the normal animal without anesthesia.

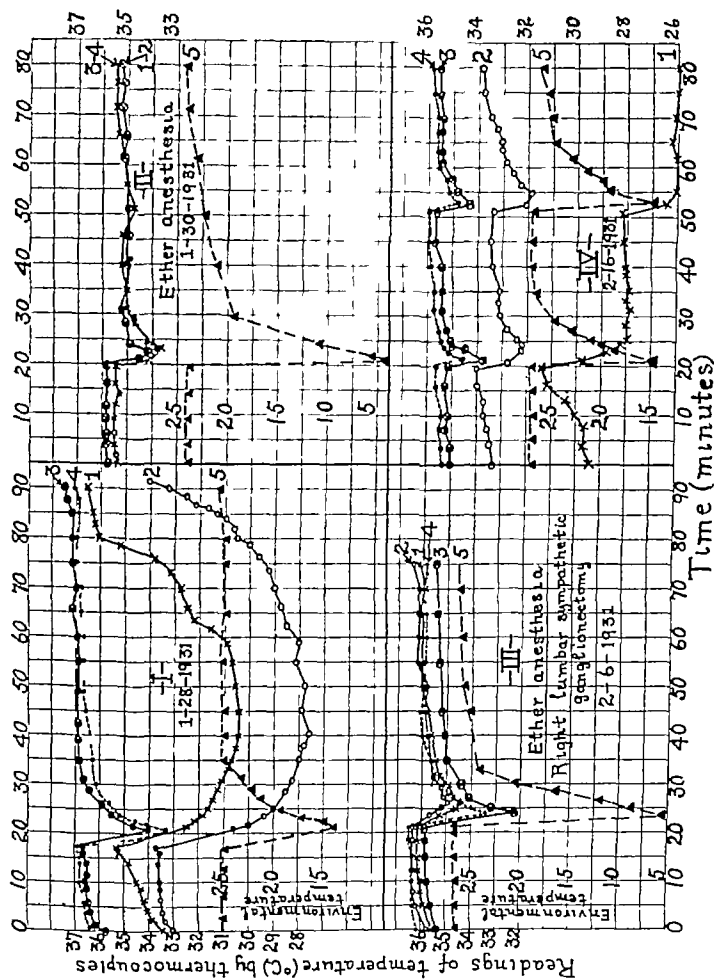


FIG 4 TIME TEMPERATURE RECORDS I, NORMAL ANIMAL WITHOUT ANESTHESIA II, UNDER ANESTHESIA III, AFTER RIGHT LUMBAR SYMPATHETIC GANGLIONECTOMY AND UNDER ETHER ANESTHESIA IV, AFTER OPERATION AND WITHOUT ANESTHESIA

In all portions of this figure, the significance of the numbers on the curves is (1) left interdigital (2) right interdigital (3) left subcutaneous (4) right subcutaneous, (5) environmental temperature, (6) right intermetatarsal, and (7) left intermetatarsal. All of these observations were made on one dog.



4 After lumbar (right or left) sympathetic ganglionectomy with trunk resection and under ether anesthesia, the time-temperature relationships of the subcutaneous, superficial and intermetatarsal temperatures closely paralleled the course of the environmental temperature but again evidenced a somewhat more rapid return to normal subcutaneous temperatures in the limbs than in the case of the normal animal without anesthesia and prior to operation

5 After lumbar sympathetic ganglionectomy and without ether anesthesia, the curves in Figures 4 (IV) and 5 (IV) definitely show that, when the environmental temperature was lowered, vasoconstriction occurred in the hind extremity not operated on so far as the superficial or

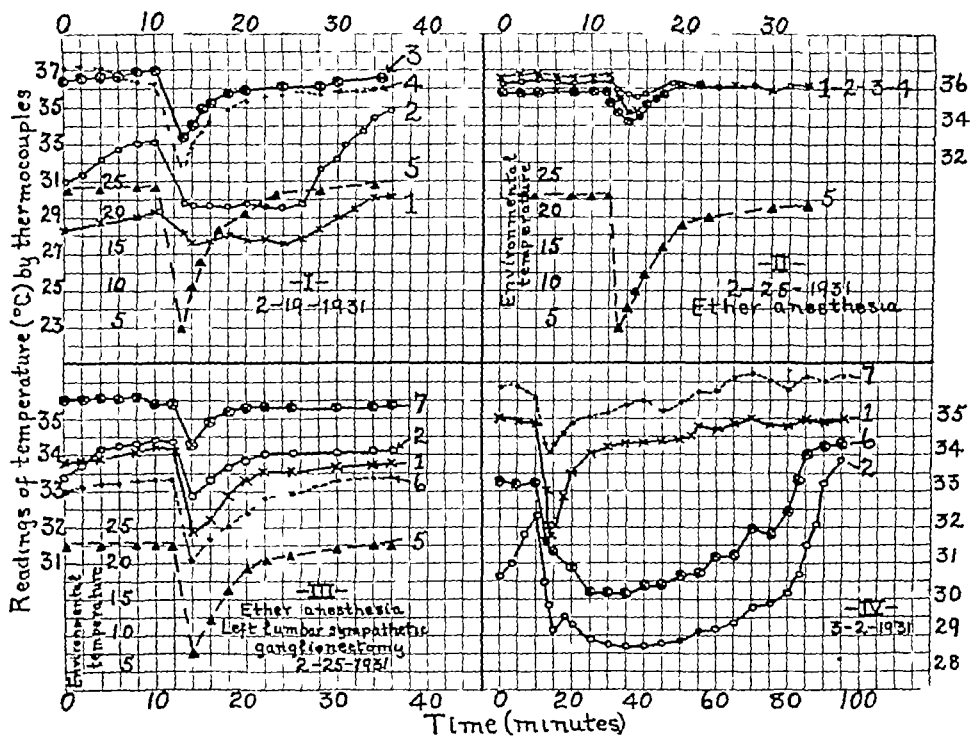


FIG 5 TIME-TEMPERATURE RECORDS OF ANOTHER DOG BEFORE AND AFTER LEFT LUMBAR GANGLIONECTOMY, WITH AND WITHOUT ANESTHESIA (FOR EXPLANATION OF NUMERALS REFER TO FIGURE 4)

intermetatarsal temperatures of the foot were concerned, but that little if any effect was produced by sympathectomy on the temperatures in the subcutaneous regions in the neighborhood of the patella

By far the greater part of our experimental work was carried out under ether anesthesia whenever an anesthetic was employed. Investigations made with amytal or procaine showed that the results obtained were similar to those secured under ether anesthesia. It seems logical to conclude

that any drug which, when administered, inhibits the functioning of the central nervous system, would produce the same results as did ether, amytal or spinal anesthesia.

Figure 6 shows the interdigital and intermetatarsal time temperature relationships (curves 1, 2, 6 and 7) for the feet of the dog in experiment 4 (right lumbar sympathetic ganglionectomy performed February 6, 1931) obtained March 25, 1931, after the administration of 50 mgm of amytal for each kilogram of body weight. Curve 5 shows the course of the

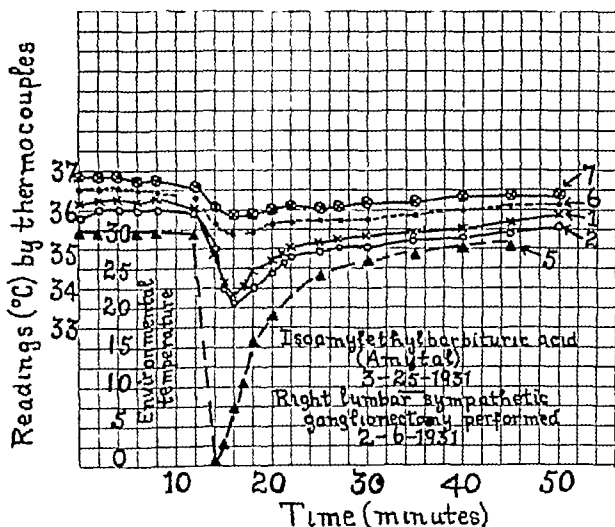


FIG. 6. TIME TEMPERATURE DATA ON THE INTERDIGITAL (CURVES 1 AND 2) AND INTERMETATARSAL (CURVES 6 AND 7) TEMPERATURES OF THE HIND EXTREMITIES OF A DOG AFTER RIGHT LUMBAR SYMPATHETIC GANGLIONECTOMY AND UNDER AMYTAL.

change of environmental temperature from 30° to - 3° C and the return within fifteen minutes to 27° C. The results are similar in every respect to those obtained on the same dog under ether anesthesia (Fig. 4 (III)).

Figure 7 shows the results obtained on the interdigital and intermetatarsal temperatures of the hind feet of a normal dog that was given an injection, April 10 1931, of 2 cc. of a 5 per cent solution of pantocaine over a period of four seconds. The injection was made between the third and fourth lumbar vertebrae. The contrast in the results obtained is more noticeable by comparison with the curves of Figures 4 (I) and 5 (I).

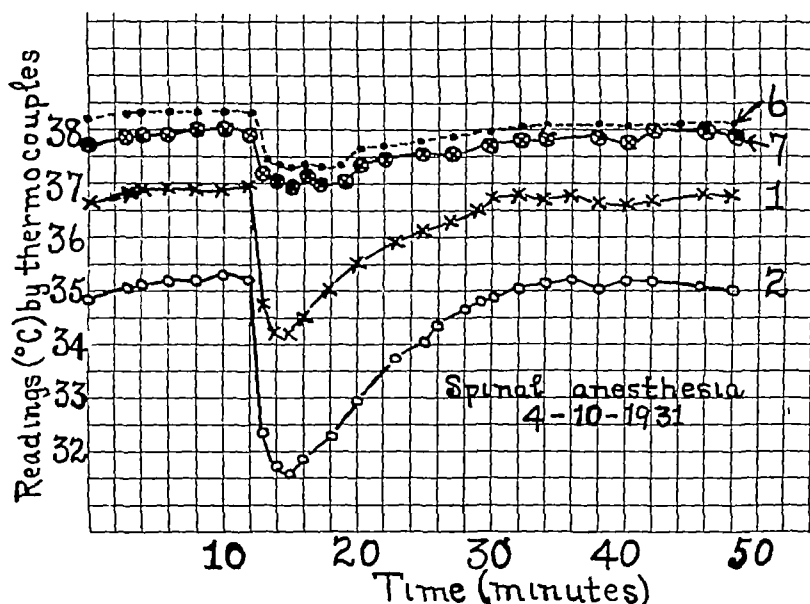


FIG 7 TIME-TEMPERATURE DATA ON THE INTERDIGITAL (CURVES 1 AND 2) AND INTERMETATARSAL (CURVES 6 AND 7) TEMPERATURES OF THE HIND EXTREMITIES OF A NORMAL DOG UNDER SPINAL ANESTHESIA

#### SUMMARY

Simultaneous measurements on the subcutaneous, interdigital and intermetatarsal temperatures of the hind extremities of dogs were made with thermocouples and a series of data was obtained regarding the thermal changes produced by environmental temperature, anesthesia and lumbar sympathetic ganglionectomy with trunk resection. The results showed that, in normal dogs, there was a marked drop in the superficial temperatures of the feet which was maintained over a considerable period when the environmental temperature of the apparatus housing the extremities was quickly lowered from temperatures of  $23^{\circ}$  to  $27^{\circ}$  C to the neighborhood of the freezing point. Subcutaneous temperatures of the limbs closely followed changes in the environmental temperature. The temperature-time relations of the intermetatarsal tissues of the feet paralleled the results obtained interdigitally.

Under ether, amytal or spinal anesthesia, superficial, subcutaneous and intermetatarsal temperatures paralleled the course of the changes in the environmental temperature and indicated absence of vasoconstriction under sudden changes in the environmental temperature.

Significant differences were not found in the course of any of the time-temperature relations following lumbar sympathetic ganglionectomy and trunk resection when the animal was under ether, amytal or spinal anesthesia. Without anesthesia, however, the time-temperature relations for the superficial and subcutaneous temperatures of the foot on the operated side showed the usual small decrease of temperature followed by

a rapid rise to normal conditions with changes in environmental temperature and indicated absence of vasoconstriction. Records of the changes of temperature of the foot on the side which was not operated on were similar to those obtained in the normal animal and indicated the presence of vasoconstriction which may persist from twenty minutes to an hour.

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## OBSERVATIONS ON THE FORMATION OF WHEELS

### IV THE INFLUENCE OF CALCIUM CONCENTRATIONS ON HISTAMINE WHEELS<sup>1</sup>

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The principal lesion of the type of allergy manifested by asthma, hay fever, and urticaria is a localized edema. This is typified in allergic urticaria wherein each wheal represents a local edema, and also in the wheal produced by a positive skin test to allergens. These wheals result from the contact between the introduced allergen and its specific antibody in the tissues. There is considerable evidence to show that when this contact occurs, histamine or a histamine like substance is formed (1). It is this substance that causes the edema. Furthermore, when histamine itself is injected intradermally the resulting wheal is clinically and morphologically identical to an allergic wheal (2), a fact which holds true for both human and dog skin. Since it is possible, therefore, to simulate allergic edema with histamine, a method is offered for study of the mechanism of this lesion under controlled conditions.

In the course of experiments which we have previously reported, indications of the existence of a substance in normal skin which is capable of influencing the size of histamine wheals were noted (3). In investigating this point, it was found that saline extracts of dog skin, when added to the histamine solution used for injection, greatly enhanced the size of the resultant wheals.

The extract was prepared as follows: a dog was anesthetized with amytal, and a section of skin removed from the abdomen, (previously de-haired with sodium sulphide). The skin was chopped and extracted in physiologic saline solution (1 gram skin to 9 cc. saline), and histamine acid phosphate added to a concentration of 1 to 10,000. After standing one to two hours at room temperature, 0.02 cc. of the supernatant fluid was injected intradermally.

The injections were made on the uninjured side of the abdomen of the same dog, and on the outer surface of the thigh. At the same time,

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<sup>1</sup> A preliminary report of this work appeared in the Proc. Soc. Exp. Biol. and Med., 1930, xxviii, 468.

similar injections were made of a 1 to 10,000 histamine<sup>2</sup> solution, to which no skin extract had been added, and of a 1 to 10 skin extract without histamine. Uniformly consistent results were obtained, and the wheals produced by the histamine-skin solution were invariably larger than those from the histamine alone. In fact, they frequently exceeded in size wheals

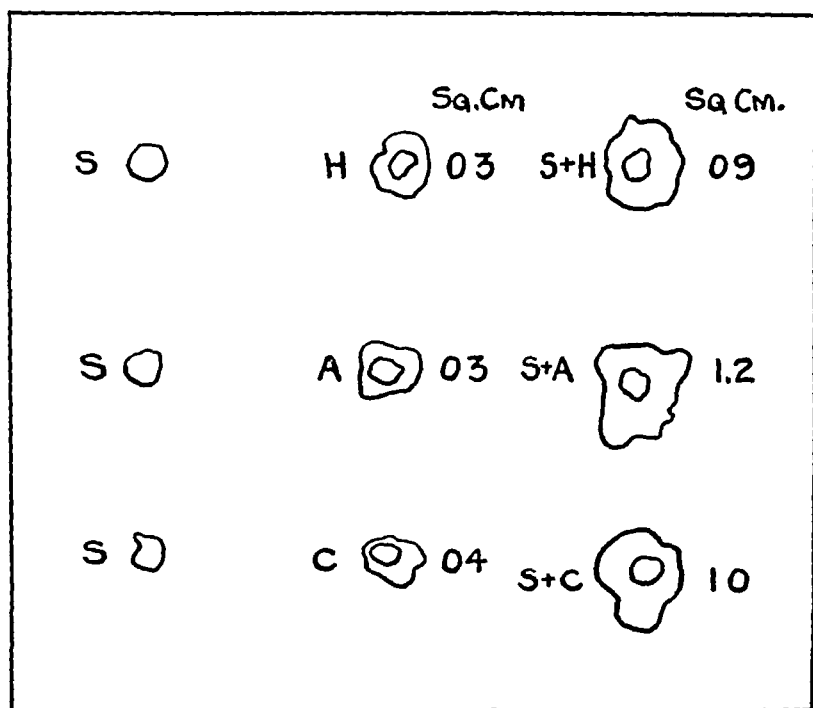


FIG 1 THE EFFECT OF SKIN EXTRACT ON HISTAMINE, ATROPINE AND CODEINE WHEELS

S = 0.02 cc of skin extract 1 to 10 dilution

H = Wheal from 0.02 cc of histamine acid phosphate 1 to 10,000 dilution

A = Wheal from 0.02 cc of atropine sulphate 1 to 1,000 dilution

C = Wheal from 0.02 cc of codeine sulphate 1 to 1,000 dilution

S+ = Wheal from 0.02 cc of skin extract 1 to 10 dilution made up to a concentration of 1 to 10,000 histamine, and 1 to 1,000 atropine and 1 to 1,000 codeine

Figures = Increase in size of wheals

In this, and in the following figures, the measurements were obtained by marking with ink the outline of the original bleb due to the injection, and the outline of the resulting wheal at the end of fifteen minutes. Tracings were made of these outlines at once and measured with a planimeter. The difference between the two areas represents the size of the wheal, which is expressed in square centimeters.

<sup>2</sup> Hereafter by 1 to 10,000 histamine solution is meant a 1 to 10,000 histamine acid phosphate solution

produced by a histamine solution 10 times as strong as that used. The skin extract alone gave no wheals. Similar results were obtained when either atropine sulphate or codeine sulphate was used instead of histamine acid phosphate as the wheal producing agent.

Table 1 compiled from the protocols of consecutive experiments is representative of the results obtained in a large series of such tests.

TABLE 1  
*The effect of skin extract on histamine wheals*

Histamine*	Histamine and skin extract†
sq. cm.	sq. cm.
0.2	0.8
0.2	0.7
0.3	0.9
0.4	0.9
0.3	0.8
0.2	0.8
0.5	0.9
0.5	1.0
0.2	0.6
0.3	0.9
0.2	0.7
0.3	0.6
0.4	0.7
0.3	0.8
0.2	0.8
0.3	0.9
0.4	1.0

\* Increase in size of wheals from 0.02 cc. histamine acid phosphate, 1 to 10,000 dilution measured in square centimeters fifteen minutes after injection.

† Increase in size of wheals from 0.02 cc. of skin extract 1 to 10 with histamine acid phosphate added to a concentration of 1 to 10,000 measured in square centimeters 15 minutes after injection. Each pair of figures represents the same experiment. No wheals occurred from the control injections of skin extract alone.

These results might be explained on the basis of Lewis' theory regarding the presence of histamine, or a histamine like substance, (H substance) in skin (1) were it not for the fact that the skin extract alone is incapable of producing a wheal, no matter how concentrated or diluted. Moreover histamine color tests by the method of Hanke and Koessler (4) were used to determine the amount, if any, of histamine present, since Harris and others have extracted histamine from the skin (5). These tests showed that histamine was present only in extracts which had decomposed slightly. When such extracts were freed from nitrogenous matter (by mercury precipitation), including any histamine or histamine like substance possibly present, their wheal enhancing activity was unimpaired. Consequently, the augmenting substance extracted from skin is evidently neither histamine nor a histamine like substance.



These results induced us to undertake a detailed chemical analysis of skin extract to determine if possible the exact nature of the augmenting substance. The various steps in the separation were, briefly, as follows.

One part of chopped dog skin was soaked in nine parts of distilled water for at least one hour, and then filtered. Two volumes of 95 per cent alcohol were added to one volume of filtrate and the precipitated proteins removed by filtration. The filtrate from this step was evaporated to dryness, at temperatures below 56° F, and the resulting residue extracted with water. A saturated mercuric acetate solution was added to this watery extract, and this caused a white flocculent precipitate which was filtered off, washed and suspended in water. Hydrogen sulphide was passed through to precipitate the mercury. This precipitate was discarded and air passed through to free the acid solution from hydrogen sulphide. Thirty per cent mercuric sulphate in 10 per cent sulphuric acid, and barium carbonate were next employed (according to the method of West and others (6)) to free the solution from practically all remaining nitrogenous compounds and phosphates. Then, sulphuric acid was added slowly until all traces of barium were removed and the solution made slightly acid. The mercury and hydrogen sulphide were removed as before, and the filtrate from this step dialysed through thick collodion sacs. The activity was found to remain on the inside of the sac. This solution was evaporated and the slight residue extracted successively with hot chloroform, hot alcohol and hot water. The chloroform and alcohol portions were evaporated and water solutions made from each of the residues. The chloroform extracted all fatty material and the activity was found only in the water extracts from the alcohol and water portions. The solutions when evaporated were found to contain a few crystals of a distinct type which appeared again and again in various extracts at this stage. These crystals resembled those of calcium sulphate. Crystals to a weight of 3.6 mgm. were obtained from 1,000 cc. of an active extract representing 100 grams of tissue, and after quantitative analysis were found to consist largely of calcium sulphate.

We learned that calcium salts would not dialyse through a thick collodion membrane and consequently a technique was employed which produced thinner sacs (7). When these thin sacs were used for dialysis of the extracts, the activity was found in the dialysate. Quantitative calcium determinations showed also that most of the calcium was in the solution outside the sac.

Several simplifications were introduced into the method of preparing the extracts. (1) Since the sodium chloride was needed only to make the solutions isotonic, it was added just before testing on the dog. This allowed us to make up the extracts and carry them through the various stages of purification in distilled water instead of in saline. (2) In earlier experiments an incubation period of several hours, after addition

of the histamine solution, appeared to be necessary to obtain the full augmentation effect. With the later, purified extracts this period could be omitted, and the histamine added just before testing. Control tests were run on each extract at every stage of the process of separation. These were made by injecting intradermally the usual 0.02 cc of the extract before adding any histamine. If the control gave a slight wheal (probably due to decomposition products of nitrogenous matters) the corresponding test, (the extract with added histamine), was discarded.

Parallel experiments were run on other tissues of the dog, and their extracts subjected to the same process of analysis. The heart, liver, lungs, muscle (both smooth and striated) were found to have more or less activity. Blood serum from both dog and man, gave just as consistent results as skin. Since blood serum was both easy to procure and to manipulate we employed it extensively. From these extracts, also, calcium sulphate crystals were isolated, and evidently the calcium present in these solutions was responsible for their augmenting effect.

As it now seemed clear that the augmenting effect was due to the calcium present in the extracts, quantitative calcium determinations were run on all the fractions obtained from the various steps of the analysis. It was found that the later, purer portions of the extract had a higher calcium content than the earlier impurer fractions. This led to the discovery that the reagents employed to precipitate the proteins present, such as mercuric acetate and barium carbonate, contained considerable calcium in impurities. Since the amount of calcium necessary to produce augmentation of histamine wheals is very minute, this gave a considerable source for error. Even the amount of calcium in ordinary filter papers was sufficient to influence the results of the experiment.

However, these steps had enabled us to eliminate practically everything but calcium from the extract, without destroying its augmenting effect. On repeating the analysis and using ashless filter papers, silica dishes for evaporating solutions, etc. active extracts were still obtained. Extracts prepared in this way had an average calcium content of 3 parts per million, a decrease of approximately 60 per cent, since no calcium impurities were added to the original extract.

Although it seemed that this amount of calcium was far too low to account for the results, solutions of pure calcium sulphate in corresponding dilutions were tested. Solutions of this salt at dilutions varying from 1 to 10,000 to 1 to 10,000,000 were found to have the same augmenting effect as the skin extract. The dilution of 1 to 100,000 usually gave the greatest augmentation. Calcium sulphate solutions in these dilutions produced no wheal except in the presence of histamine.

At an early stage in these experiments, ashing of the skin extract had been tried as a method for removing the organic material, but it was found that this procedure completely destroyed its activity. If the ash con-

tained a high calcium concentration a solution prepared from it remained inactive. But when this solution was diluted to the optimum calcium concentration (1 to 100,000) the augmentation effect was again apparent

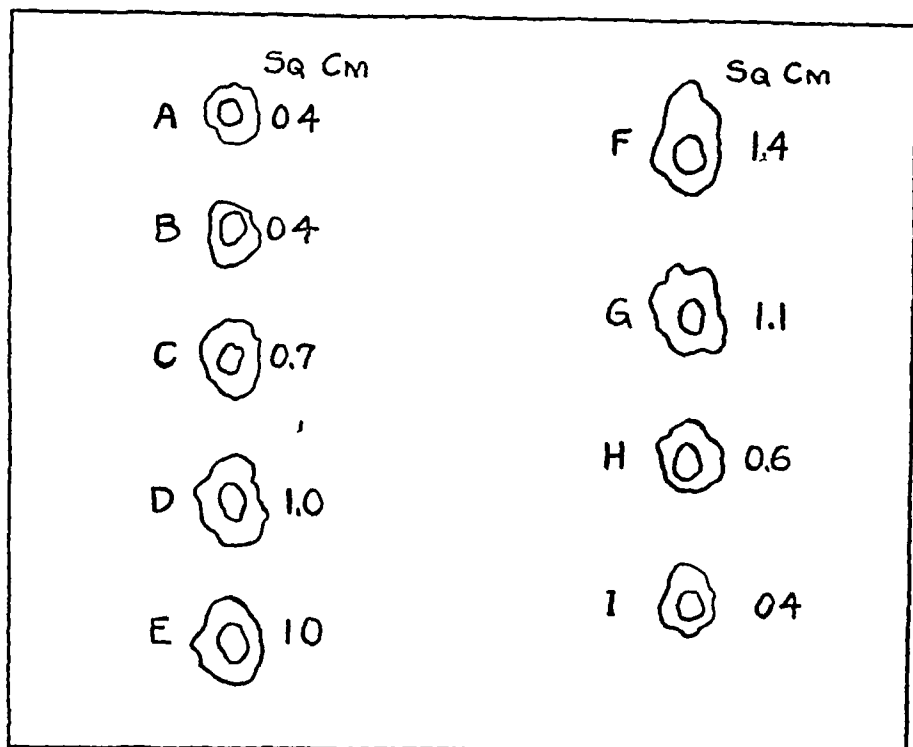


FIG. 2 THE EFFECT OF CALCIUM CONCENTRATIONS ON HISTAMINE WHEELS

A = Histamine acid phosphate solution 1 part to 10,000

B =  $\text{CaSO}_4$  1400 parts per million\*

C =  $\text{CaSO}_4$  700 parts per million

D =  $\text{CaSO}_4$  100 parts per million

E =  $\text{CaSO}_4$  50 parts per million

F =  $\text{CaSO}_4$  10 parts per million

G =  $\text{CaSO}_4$  7 parts per million

H =  $\text{CaSO}_4$  0.8 part per million

I =  $\text{CaSO}_4$  0.08 part per million

At times ashing was substituted for the mercury precipitation of proteins. In weak solutions this improved their augmenting effect, since no calcium was lost in this procedure.

These results showed that the lack of augmenting power in an extract, or in a fraction of such an extract obtained during its analysis, might be

\* All  $\text{CaSO}_4$  solutions were made up to contain histamine acid phosphate 1 part to 10,000. Dilutions refer to calcium sulphate  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ . The calcium forms approximately one-fourth of the total.

due to an excess of calcium, rather than to its absence. Henceforth, all skin tests on the dog were made in triplicate, (a) at the usual concentration of one gram of skin to 9 cc. saline, (b) at one tenth this concentration, and (c) at varying strengths of 3 to 7 times the usual concentration.

Although there was little doubt in our minds that calcium was responsible for all the augmenting effect, various other possibilities were considered. We found that strong solutions of phosphates also possessed

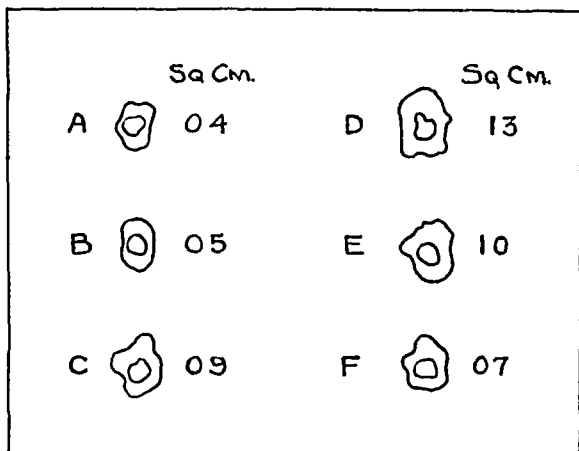


FIG. 3 THE EFFECT OF CONCENTRATIONS OF SKIN EXTRACT ON HISTAMINE WHEELS

- A = Histamine acid phosphate solution 1 part to 10,000
- B = 1 part skin extract diluted 1.5 times with saline\*
- C = 1 part skin extract diluted 5 times with saline
- D = 1 part skin extract diluted 10 times with saline
- E = 1 part skin extract diluted 100 times with saline
- F = 1 part skin extract diluted 200 times with saline

considerable augmenting power. In none of our extracts, however, was the phosphate present in sufficient concentration to have this effect. The phosphate contents were determined colorimetrically according to the method of Fiske and Subbarow (8), and were always much lower than the concentrations of sodium phosphate which are capable of augmenting histamine wheals.

One factor which had been constant throughout these experiments was the amount of sodium chloride which was added to produce isotonicity in

\* All skin extract solutions were made up to contain histamine acid phosphate 1 part to 10,000

the solutions just before testing on the dog. At this stage glucose was substituted for sodium chloride in order to exclude any effect which the saline solution may have had. The same results were obtained as when saline was used.

As the histamine used was in the form of an acid phosphate, this was replaced by an equivalent amount of histamine di-chloride to determine whether or not the negative radical had any effect. With histamine di-chloride in dilution of 1 to 16,600, comparable results were obtained, and the augmentation was just as great and just as consistent as when histamine acid phosphate in dilution of 1 to 10,000 was used.

It was possible to inhibit the augmenting effect of skin extract on histamine in various ways. If the pH of the solutions was much more acid, or alkaline, than that of blood, the augmentation was decreased. Therefore, the pH was always adjusted to approximately 7.2 (using phenol red as the indicator), before testing on the dog, to insure obtaining the maximum augmentation.

The addition of strong soap solutions completely destroyed the activity of the extracts, and even cut down to a considerable degree the size of the standard histamine wheal. Sodium citrate solutions had a similar effect, possibly due to their power of changing the diffusibility of calcium (9). As stated above, too high a calcium concentration prevented augmenting effect. This held true also when calcium was added to an extract that was already active.

A 10 per cent solution of calcium chloride (10) will of itself induce a wheal not unlike that from histamine. This, however, is so far beyond the concentrations used in these experiments that the augmenting effect of calcium on histamine cannot be considered as merely an added effect.

#### SUMMARY

From these experiments, it appears that calcium possesses the power of enhancing the size of wheals due to histamine (or to atropine or codeine), but only in a definite zone of concentration. The optimum concentration for different dogs, however, varies considerably. In some the 1 to 10,000 dilution gave greater augmentation than the 1 to 1,000,000 dilution, in others this effect was reversed. In most instances the optimum dilution was 1 to 100,000. Outside of these limits, calcium seems incapable of increasing the size of wheals. The explanation for this is not clear. Further experiments are being carried out to study the behavior of wheals under conditions that are supposed to influence the concentration of calcium in the fluids and tissues of the body.

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Endermic Reactions I



## OBSERVATIONS ON THE FORMATION OF WHEELS

### V THE EFFECTS OF VARIATION OF THE CO<sub>2</sub> COMBINING POWER OF THE BLOOD ON HISTAMINE WHEELS

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(Received for publication August 14, 1931)

In a previous paper (1) it was shown that calcium salts in very high dilutions only are able to augment the size of histamine wheals. The following experiments were designed to show the effects of changes in CO<sub>2</sub> combining power in the blood of dogs after the administration of acid and alkaline salts, on the size of histamine wheals. The method employed was as follows:

Control tests were made intradermally with the following solutions: 0.02 cc. of histamine acid phosphate, 0.02 cc. of a 1 to 100,000 dilution of calcium sulphate, and 0.02 cc. of a 1 to 10 dilution of active skin extract. Each of the three solutions were made up to contain the same concentration of histamine acid phosphate. The resulting wheals were measured 15 minutes after intradermal injections, and were recorded in square centimeters. Both calcium and skin extract were used in these experiments to serve as a check on each other, since it has been shown that the augmenting power of skin extract on histamine wheals is due to its calcium content.

Blood was then drawn under oil from the heart. The serum was separated and exposed to a CO<sub>2</sub> (5 per cent) O<sub>2</sub> (95 per cent) mixture, and the CO<sub>2</sub> combining power determined according to the method of Van Slyke and Neill (2). Ammonium chloride dissolved in 40 times its weight in water to prevent vomiting was administered by stomach tube. Six grams of the salt were usually sufficient to produce a distinct lowering of the CO<sub>2</sub> combining power of the blood in a dog of 5 kilos. At intervals of 15 to 30 minutes after the ammonium chloride had been given, intradermal tests, with the same solutions as above, were repeated. As soon as a variation in the size of wheals when compared to the control tests was noted, blood was drawn again and the CO<sub>2</sub> combining power of the serum determined as before. If the drop in CO<sub>2</sub> was pronounced, the intradermal tests were repeated again 18 hours later, and a final CO<sub>2</sub> estimation made. No anesthetic was used.

When the CO<sub>2</sub> combining power of the blood was lowered, the sizes of the wheals were always smaller than the controls, although the amount



of total calcium in the blood serum remained unchanged. The amount of lowering of the CO<sub>2</sub> combining power varied considerably, but with a drop of only 12 volumes per cent, the sizes of the wheals at times were markedly reduced when compared with the control tests. As the CO<sub>2</sub> combining power gradually returned to normal, the wheals again became larger. For example, in one experiment, a histamine wheal measured 0.7 sq. cm. when the CO<sub>2</sub> combining power of the blood was 60 volumes per cent. After the administration of ammonium chloride, the CO<sub>2</sub> dropped to 45 volumes per cent. A histamine wheal then measured but 0.4 sq. cm. Finally, as the CO<sub>2</sub> rose to about its previous level, a histamine wheal again measured approximately 0.7 sq. cm. In these experiments

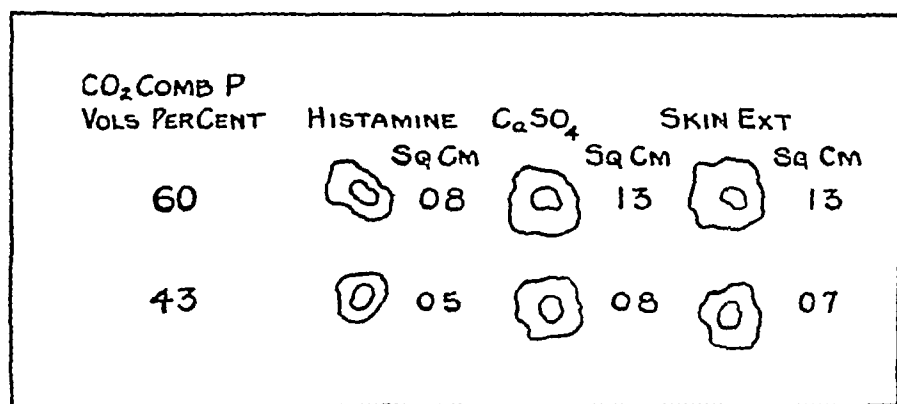


FIG. 1 THE EFFECT OF A LOWERED CO<sub>2</sub> COMBINING POWER OF THE BLOOD ON THE SIZE OF WHEELS

Histamine = Histamine acid phosphate 1 to 2,000 dilution

CaSO<sub>4</sub> = 1 to 100,000 dilution made up to contain histamine acid phosphate 1 to 2,000

Skin extract = 1 to 10 dilution made up to contain histamine acid phosphate 1 to 2,000

Figures = Increase in area of wheals measured in square centimeters fifteen minutes after injection

histamine acid phosphate was used in high concentrations so that the resulting wheals were large. This was done in order to measure variations in size more accurately. In preliminary experiments the pH of the blood was found to be lowered after the administration of acid salts associated with a diminution in size of wheals. The pH was measured according to the method of Hastings and Sendroy (3).

The same method was followed in experiments to determine the size of wheals when the CO<sub>2</sub> combining power of the blood was elevated. A distinct rise in CO<sub>2</sub> was usually obtained with 200 cc. of a six per cent solution of sodium bicarbonate administered by stomach tube.

When CO<sub>2</sub> combining power was elevated, there was always an associated increase in the sizes of wheals as compared to the controls.

Moreover, as the  $\text{CO}_2$  resumed its original level, the wheals again became smaller. For example, a histamine wheal measured 10 cc. when the  $\text{CO}_2$  combining power was 60 volumes per cent. Forty five minutes after sodium bicarbonate had been given, the  $\text{CO}_2$  value rose to 78. The total serum calcium remained unchanged. At this time, a histamine wheal measured 16 cm., about 60 per cent larger than the control wheal. The following day, both the  $\text{CO}_2$  combining power and the size of the histamine wheal had assumed their original values. In preliminary experiments, the pH of the blood was found to be elevated after the administration of alkaline salts associated with an increase in the size of wheals.

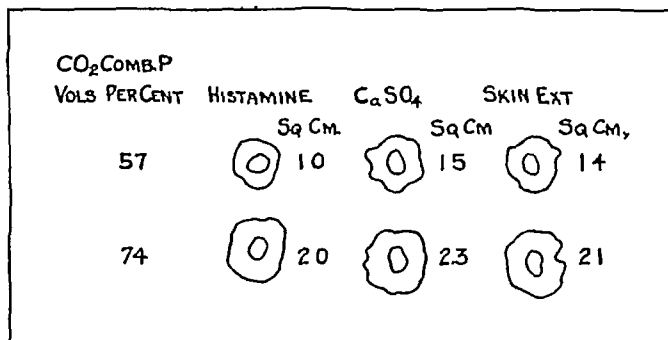


FIG II THE EFFECT OF AN ELEVATED  $\text{CO}_2$  COMBINING POWER OF THE BLOOD ON THE SIZE OF WHEELS

Histamine = Histamine acid phosphate 1 to 2,000 dilution

$\text{CaSO}_4$  = 1 to 100,000 dilution made up to contain histamine acid phosphate 1 to 2,000

Skin extract = 1 to 10 dilution made up to contain histamine acid phosphate 1 to 2,000

Figures = Increase in area of wheals measured in square centimeters fifteen minutes after injection

The following protocol shows the results of two experiments, one with a lowered and one with a raised  $\text{CO}_2$  combining power. These were selected as typical experiments from a series of 19 (Table I).

It is known that citrates markedly influence diffusible and ionic calcium in complex solutions such as blood (4). This (5) was suggested in previous experiments (1). To a 1 to 100,000 solution of calcium sulphate, containing four per cent sodium citrate, was added histamine acid phosphate, in an amount sufficient to make a concentration of 1 to 10,000. The mixture was adjusted to a pH of 7.2. When injected intradermally into a normal dog, calcium

TABLE I

*The effects of changes in CO<sub>2</sub> combining power of the blood on the sizes of wheals*

Date, dog number and weight	Time	CO <sub>2</sub> combining power	Serum calcium	Histamine	CaSO <sub>4</sub>	Skin extract	Comment
	a m	volumes per cent	per 100 cc.	sq cm	sq cm		
March 18, 1931 Dog 5 Weight approximately 5 kilos	10 00	60	10 3	0 8	1 3	1 1	6 0 grams NH <sub>4</sub> Cl in 200 cc water given in stomach
	10 15						
	10 30			0 9	1 2	1 0	
	10 45	47	10 2	0 5	0 6	0 7	
April 5, 1931 Dog 9 Weight approximately 7 kilos	11 00	59	9 8	1 0	1 4	1 3	6 0 grams NaHCO <sub>3</sub> in 200 cc water given in stomach
	11 15						
	11 30			1 2	1 4	1 3	
	11 45	78	10 0	1 6	1 8	1 9	

Histamine = Histamine acid phosphate 1 to 2,000 dilution

CaSO<sub>4</sub> = 1 to 100,000 dilution made up to contain histamine acid phosphate 1 to 2,000

Skin extract = 1 to 10 dilution made up to contain histamine acid phosphate 1 to 2,000

Figures = Increase in area of wheals measured in square centimeters fifteen minutes after injection

histamine wheal Moreover, a histamine solution containing four per cent sodium citrate and no calcium induced a wheal that was smaller than that given by histamine alone

The effect on wheal formation of sodium citrate administered to a dog, was studied One hundred cc of a five per cent sodium citrate solution was given by stomach tube The same procedure was followed as that recorded in the experiments above It was found that sodium citrate invariably caused a rise in the CO<sub>2</sub> combining power Despite this, in no instance did the size of wheals appreciably increase As a rule, they remained about the same size as the controls Occasionally there was a decrease In one dog on which the experiment was done repeatedly, there was a marked reduction (about 40 per cent) in the size of wheals after sodium citrate had been given, although the CO<sub>2</sub> combining power of the blood rose 20 volumes per cent The total serum calcium was not appreciably affected

That neither the sodium nor the chloride ion of the salts administered was responsible for the changes noted, had been determined in previous experiments wherein glucose was substituted for the sodium chloride used to produce isotonicity in the solutions injected Under these circumstances, the wheal-forming activity of the solutions remained unchanged

## SUMMARY

1 The ingestion of acid in the form of ammonium chloride diminishes the sizes of histamine wheals as well as those formed from a combination of histamine with calcium sulphate and from histamine with skin extract. Ingestion of sodium bicarbonate, on the other hand, increases the size of such wheals.

2 The ingestion of sodium citrate caused an alkalization which was not accompanied by an increase in size of histamine wheals. At times the wheals were markedly reduced in size.

While it is tempting to consider that these phenomena may be explained on the basis of changes in calcium ion concentration, the conditions actually existing in the tissues are too little known to justify this conclusion.

Further experiments are being conducted on the behavior of the localized edemas of allergy under conditions that influence wheal formation.

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# STUDIES OF CALCIUM AND PHOSPHORUS METABOLISM

## VI IN HYPOPARATHYROIDISM AND CHRONIC STEATORRHEA WITH TETANY WITH SPECIAL CONSIDERATION OF THE THERAPEUTIC EFFECT OF THYROID

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(Received for publication August 3 1931)

Following the observation by Aub, Bauer, Heath, and Ropes (1), that the thyroid hormone exerts a marked effect upon the excretion of calcium, it became of interest to determine the therapeutic effect of this internal secretion upon the calcium metabolism in tetany. In hyperthyroidism, although the blood calcium and phosphorus levels are essentially normal, the calcium and phosphorus excretions are abnormally high. Tetany of the low calcium variety, however, has been shown to have an abnormally low calcium excretion associated with the abnormally low blood calcium level. The primary purpose of these metabolic studies was to study the influence of thyroid medication on the level of calcium and phosphorus in the blood and excreta of patients suffering from tetany. Other observations, however, were made for comparison and this paper includes data illustrating the influence on inorganic salt metabolism of

- 1 Hypoparathyroidism,
- 2 Chronic steatorrhea complicated by tetany,
- 3 The immediate and prolonged use of parathyroid and thyroid medication in the tetany of hypoparathyroidism, and
- 4 The production of acidosis in the above types of tetany

The data reported in this paper were obtained from three cases of tetany which we observed over prolonged periods. K L (Case I) had severe, chronic parathyroid tetany which was precipitated by two radical thyroidectomies for very mild Graves disease. The tetany eventually could not be controlled, and the patient died. B W (Case II)<sup>1</sup> apparently had idiopathic parathyroid tetany. DeLaB (Case III),<sup>1</sup> a young woman with tetany produced by celiac disease or steatorrhea, was similar to patients reported by Blumgart (2), Thaysen (3), Holmes and Starr (4), Linder and Harris (5), and Hunter (6). The summaries of the case histories are attached to the end of this paper. These cases were studied in

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<sup>1</sup> Cases II and III are further discussed in other papers (9, 10, 11)

the metabolism ward of the Massachusetts General Hospital with the same careful routine and methods already fully described in papers I and II of this series (7) (8) We were able to maintain a rigid regime with constant food intake in which a change of medication was often the only variant The excellent cooperation of the patients allowed us to obtain repeated observations over many months (The metabolic data are given in Tables I, II, and III)

*The metabolic characteristics of parathyroid tetany*

These three patients grouped themselves into two types K L and B W (Cases I and II) represented parathyroid deficiencies, while DeLaB represented a metabolic abnormality which was probably primarily digestive Each of the types had a low blood calcium level and the signs and symptoms of tetany (see Table IV) The difference in these two types was best seen in the blood inorganic phosphorus levels, a determination which is of prime importance in the differentiation of types of tetany Thus, both K L and B W had elevated blood phosphorus levels, characteristic of parathyroid tetany, while DeLaB had a lower level than normal

Low blood calcium values in parathyroid tetany are now well established, and these cases showed a reduction which was more than 50 per cent below the normal value Calcium chloride intravenously did not raise the blood calcium or affect blood phosphorus levels for any prolonged period Within two hours after the injection these values had returned to their previous levels in Case I Hourly blood calcium determinations, after B W (Case II) ingested 5 grams of calcium lactate, disclosed a maximum elevation at the end of two hours of less than 1 mgm

The extraordinarily low calcium excretion in the urine, like the low serum calcium, was present in both types of tetany In the three cases it averaged only 26 mgm in three days while in our normal controls the average was 190 mgm The fecal excretion on a low calcium diet was essentially normal in the two patients with hypoparathyroidism in contrast to the slight elevation in the patient with steatorrhea This indicates that the excretion of calcium by bowel, inasmuch as it is not decreased by a low blood calcium, is probably not a threshold phenomenon This supposition is strengthened by the finding of a decreased fecal calcium excretion in hyperparathyroidism

The excretion of phosphorus in the two untreated cases of parathyroid tetany was lower than that in normal individuals Just as with calcium, the urinary phosphorus excretion was reduced, but the fecal excretion was essentially normal Thus, just as a high partition of phosphorus in the urine as compared with the feces is characteristic of hyperparathyroidism (12), the opposite is the case in parathyroid tetany

TABLE I  
Katherine L aged 25, white female Admitted January 28, 1926  
(Intake and output per 3-day period)

Date ending period	Re- c- ord num- ber	Calcium				Phosphorus				Nitrogen				Blood plasma				Date	Ba- se- line bolle ratio	Treatment and remarks	
		Excretion			In- take	Excretion			In- take	Excretion			In- take	To- tal ex- crete in- take	Date	Ca	P				Se- rum Ca
		Urine	Feces	Total		Urine	Feces	Total		Urine	Feces	Total									
2/12	1	.04	78	82	23	.25	78	124	1.20	7.2	1.5	8.8	14.2	2925	2/12	4.3	5.6	4.8	2/1	-11	2/11 Severe tetany CaCl <sub>2</sub> 1 gram intra- venously, plus parathormone 20 units 2/12 to 2/16 Parathormone 10 units daily 2/16 Severe tetany, CaCl <sub>2</sub> 1 gram intra- venously plus parathormone 30 units 2/17 Parathormone 15 units daily until 4/7
2/15	2	.01	14	15	21	.50	36	93	1.31	7.5	1.9	9.4	13.3	4130	2/15	4.3	5.8		2/2	-12	
															2/9	4.4	4.8		2/3	-18	
2/21	3	.01	29	30	33	.48	21	59	1.43	11.6	2.0	13.6	15.0	5745	2/18	4.6	5.7	4.3	2/4	-20	3/2 Thyroid 10 mgm. 3/6 - 2 3/4 Attacks of tetany until now Nose bare- 3/9 - 10 3/4 Thyroid 10 mgm. 3/10 Thyroid 0.3 gram daily 3/12 Thyroid 5 mgm. 3/16 +22 3/17 Thyroid stopped
2/24	4	.04	33	37	33	.98	70	(1.08)	1.48	10.0	4.3	14.3	15.8	5745	2/22	5.0	4.2	4.3			
2/27	5	.15	34	40	33	.96	12	1.08	1.63	17.1	2.1	19.2	20.1	6281	2/21	5.6	4.3	4.3			
3/2	6	.06	20	30	33	1.13	13	1.26	1.63	14.8	1.8	16.6	20.1	6333	3/4	7.8	6.0	4.3	3/6	-2	3/8. Attacks of tetany until now Nose bare- 3/9 - 10 3/4 Thyroid 10 mgm. 3/10 Thyroid 0.3 gram daily 3/12 Thyroid 5 mgm. 3/16 +22 3/17 Thyroid stopped
3/5	7	.06	47	53	33	74	85	1.59	1.48	10.4	5.7	16.1	18.0	4810	3/6	8.0	4.5	4.3			
3/9	8	.07	32	39	31	1.60	53	2.48	1.52	21.4	2.5	23.9	19.3	5575	3/8	8.0	4.5	4.3			
3/12	9	.14	40	54	33	1.60	33	1.93	1.63	20.1	1.5	21.6	20.1	6333	3/12	8.5	4.0	4.3	3/9	-10	3/8. Attacks of tetany until now Nose bare- 3/9 - 10 3/4 Thyroid 10 mgm. 3/10 Thyroid 0.3 gram daily 3/12 Thyroid 5 mgm. 3/16 +22 3/17 Thyroid stopped
3/14	10	.17	34	51	33	2.10	28	2.36	1.63	24.3	2.7	27.0	20.1	6333	3/15	10.1	4.4	4.3			
3/17	11	.33	53	57	33	2.17	43	2.60	1.63	26.7	3.4	30.1	20.1	6633	3/17	11.2	4.3	4.3			
3/20	12	.75	48	123	33	1.61	40	1.91	1.63	20.5	2.3	22.8	20.1	6333	3/22	9.5	2.5	4.4	3/18	+22	3/20 Thyroid 10 mgm. 3/23 +5 3/26 10.7 4.6 3/27 -8 3/30 11.0 4.7 4/2 -16
3/23	13	.74	53	126	33	1.98	40	1.40	1.63	18.5	1.9	20.4	20.1	6333	3/24	11.4	5.3	4.3			
3/26	14	.60	47	107	33	1.31	40	1.61	1.63	13.3	3.8	16.0	20.1	6333	3/26	10.6	4.7	4.3			
3/29	15	.60	54	114	33	1.11	43	1.54	1.63	15.3	2.3	15.3	20.1	6333	3/29	10.6	4.7	4.3			
4/1	16	.48	87	135	33	.84			1.63	19.1	3.9	23.0	20.1	6333	4/2	9.9	4.5	4.3			



TABLE I (continued)

Date end ing pe- riod	Calcium			Phosphorus			Nitrogen				To- tal en- er- gy in- take	Blood plasma				Ba- sal me- ta- bolic rate	Treatment and remarks
	Excretion			Excretion			Excretion					Date	Ca mgm per 100 cc	P mgm per 100 cc	Se- rum Ca mgm per 100 cc		
	Urine	Feces	Total	Urine	Feces	Total	Urine	Feces	Total								
	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	cal- ories						
4/4	35	37	72	33	72	37	109	163	138	23	151	201	0.33	4/5	0.3	47	4/7 Parathormone dosage changed,—given on alternate days only
4/7	40	61	101	33	124	61	185	163	0	25	115	201	0.33	4/7	0.4	38	
4/10	37	51	88	31	81	48	120	183	128	22	150	233	0.071	4/10	0.5	45	
4/13	21	47	68	33	46	47	92	192	103	43	206	249	0.290	4/13	0.4	50	4/13 Parathormone units 7 daily until 6/19
4/16	14	50	73	33	117	60	177	192	174	28	202	249	0.290	4/16	0.5	53	
4/19	22	13	60	73	85	61	139	192	100	28	227	320	0.290	4/19	0.7	53	4/19 Tetany Thyroxin 10 mgm Ca lac- tate 2 grams milk 100 cc
4/22	12	66	78	71	117	48	105	195	252	40	292	249	0.066	4/22	0.5	70	
4/25	13	59	72	33	113	40	162	192	247	20	276	249	0.290	4/25	0.5	71	
4/28	26	60	77	23	220	43	263	144	262	21	283	208	0.426	4/28	101	40	4/27 Thyroxin 0.5 mgm 5/1 Thyroxin 10 mgm.
5/1	21	40	67	31	125	100	225	170	100	23	102	283	0.075	5/3	70	0	
5/4	17	50	67	33	112	46	158	192	252	20	272	283	0.037	5/5	92	43	5/10 A little urine was lost
5/7	38	50	87	33	159	41	200	192	204	20	224	249	0.037	5/7	87	97	
5/10	20	68	88	130	170	01	234	202	242	33	275	245	0.045	5/9	96	9.8	
5/13	30	52	77	120	160	71	232	202	193	37	230	232	0.074	5/12	82	48	0/13 — 1 5/17 — 4 5/19 — 11
5/16	31	62	93	33	161	52	213	202	172	44	216	235	0.074	5/14	92	5.2	
5/19	32	62	94	109	143	37	201	240	146	20	175	250	0.032	5/10	78	48	
5/22	33	62	94	110	143	55	160	240	171	24	195	297	0.048	5/21	90	48	0/8 Blood nonprotein nitrogen 29
5/26	31	40	58	101	142	45	187	248	103	33	102	291	0.028	5/20	73	93	
5/28	35	58	93	91	134	59	193	249	170	27	203	288	0.028	5/28	83	93	
5/31	30	58	91	91	134	59	193	249	170	27	203	288	0.028	5/29	95	67	0/15 Unrestricted diet
6/3	37	60	97	92	138	64	172	240	194	22	232	288	0.028	5/31	88	68	
6/6	38	60	96	96	162	67	229	240	190	32	220	288	0.028	6/3	87	50	
6/10	39	37	70	107	113	63	170	240	169	53	221	288	0.028	6/6	77	47	0/12 40 6/15 41
6/12	40	33	75	108	113	63	170	240	169	53	221	288	0.028	6/7	101	45	
6/15	41	33	75	108	105	61	280	170	170	50	229	288	0.028	6/12	95	48	
														6/13	92	53	

TABLE I (continued)

Date	Re- spon- ding pe- riod	Calcium			Phosphorus			Nitrogen				Blood plasma				Date	Basal me- tabo- lic rate	Treatment and remarks	
		Excretion		In- take	Excretion		In- take	Excretion		In- take	Total	mgm. per 100 cc.	Ca	P	mgm. per 100 cc.				
		Urine	Feces		Urine	Feces		Urine	Feces										Total
6/18	42	57	76	133	2.09	1.11	3.20	20.4	9.17	29.5	31.5	0/10	7.3	5.2	7.9	cc.	6/19	Thyroid started	
6/21	43	39	114	153	3.14	1.16	4.30	20.4	4.8	25.2	31.5	6/21	8.4	4.0	8.4	cc.	6/23	-6	
6/24	44	40	92	132	4.35	1.24	5.59	20.4	6.4	26.8	31.5	6/23	7.3	5.3	7.3	cc.	6/26	-10	
6/27	45	41	93	131	2.16	1.01	3.17	20.4	8.6	29.0	31.5	6/26	8.7	5.0	8.7	cc.	11/8	-3	
11/11	46	57	154	154	2.24	1.14	3.38	22.3	2.8	25.0	24.6	11/8	4.5	5.3	4.5	cc.	11/8	Parathormone 15 units daily	
11/17	47	56	150	150	2.25	1.03	3.28	22.3	2.9	25.1	24.6	11/13	5.0	5.3	5.0	cc.	11/8	Parathormone 15 units daily	
11/20	48	58	147	147	2.25	1.00	3.25	22.5	2.4	24.9	24.6	11/17	4.9	5.6	4.9	cc.	11/10	-12	
11/23	49	10	126	136	2.26	.93	3.19	23.4	3.8	27.2	28.3	11/19	5.2	6.1	5.2	cc.	11/10	-12	
11/26	50	10	113	123	2.25	1.13	3.38	21.7	6	27.7	28.3	11/22	5.0	6.1	5.0	cc.	11/26	0	
11/29	51	55	125	137	2.25	1.17	3.42	20.0	2.0	22.0	24.3	11/27	4.4	5.8	4.4	cc.	11/29	HCl 35 cc. per day	
12/2	52	50	123	132	2.25	1.23	3.47	21.1	2.7	23.8	24.3	11/30	5.0	6.4	5.0	cc.	12/3	Milk	
12/5	53	50	123	132	2.25	1.23	3.47	20.8	2.9	23.7	24.3	12/3	4.9	6.4	4.9	cc.	12/3	NH <sub>4</sub> Cl 8 grams per day	
12/8	54	57	132	142	2.25	1.32	3.57	21.2	3.9	25.1	24.3	12/6	5.1	5.7	5.1	cc.	12/11	HCl stopped. NH <sub>4</sub> Cl 6 grams per day	
12/11	55	16	125	137	2.25	1.04	3.29	20.5	2.0	22.5	24.6	12/11	5.5	4.1	5.5	cc.	12/11	HCl stopped. NH <sub>4</sub> Cl 6 grams per day	
12/14	56	16	124	136	2.24	1.08	3.32	21.0	3.9	24.9	24.6	12/15	4.7	4.7	4.7	cc.	12/21	Parathormone 50 units	
12/17	57	18	124	142	2.24	1.17	3.41	20.5	2.6	23.1	24.6	12/21	4.9	6.2	4.9	cc.	1/3	Parathormone 30 units	
12/20	58	18	123	141	2.25	1.11	3.36	20.5	1.1	21.6	24.6	12/21	5.2	6.5	5.2	cc.	1/3	Parathormone 40 units	
12/23	59	17	121	138	2.25	1.05	3.30	20.5	1.1	21.6	24.6	12/21	4.9	6.2	4.9	cc.	1/3	Parathormone 80 units	
12/26	60	17	121	138	2.25	1.05	3.30	20.5	1.1	21.6	24.6	12/21	4.9	6.2	4.9	cc.	1/3	Parathormone 100 units.	
1/10	61	17	121	138	2.25	1.05	3.30	20.5	1.1	21.6	24.6	12/21	4.9	6.2	4.9	cc.	1/3	Parathormone 100 units.	
1/13	62	17	121	138	2.25	1.05	3.30	20.5	1.1	21.6	24.6	12/21	4.9	6.2	4.9	cc.	1/3	Parathormone 100 units.	
1/16	63	17	121	138	2.25	1.05	3.30	20.5	1.1	21.6	24.6	12/21	4.9	6.2	4.9	cc.	1/3	Parathormone 100 units.	
1/19	64	15	141	156	2.25	1.25	3.50	23.4	2.5	25.9	24.6	1/13	4.6	7.0	4.6	cc.	1/7	NH <sub>4</sub> Cl stopped	
1/22	65	15	141	156	2.25	1.25	3.50	23.4	2.5	25.9	24.6	1/13	4.6	7.0	4.6	cc.	1/21	CaCl <sub>2</sub> intravenously	
1/25	66	15	141	156	2.25	1.25	3.50	23.4	2.5	25.9	24.6	1/13	4.6	7.0	4.6	cc.	1/21	CaCl <sub>2</sub> intravenously	



TABLE II (continued)  
Second admission—October 11 1943

Date ending period	Body weight	Calcium			Phosphorus			Nitrogen			Total caloric intake	Date	Blood plasma		Basal metabolic rate	Treatment and remarks	
		Excretion		In-take	Excretion		In-take	Excretion		In-take			Ca	P			
	kilograms	Urine	Feces	Total	Urine	Feces	Total	Urine	Feces	Total	grams	grams	grams	mgm. per 100 cc.	mgm. per 100 cc.	per cent	
10/15	55.1																
10/17	55.5	.05		5.06	1.85			6.42	30.4	2.1	32.5	7390	10/17	6.0	5.5	-22	
10/21	56.2	.04		6.01	1.88			6.47	26.3	2.9	29.2	7404	10/19	6.0	5.5	-14	10/20. Thyroxin, 10 mgm.
10/23	57.2	.02		6.05	2.59			6.47	35.3	2.6	37.9	7396	10/21	5.9	6.3	-9	10/24. Thyroxin, 10 mgm.
10/27	56.6	.04		5.81	2.06			6.31	33.1	2.4	35.5	7332	10/23	5.7	5.3	-18	
10/29	57.0	.04		5.82	2.32			6.41	33.3	2.6	35.9	7356	10/27	5.8	5.4	-7	
11/1	57.5	.06		6.01	1.79			6.46	30.1	2.5	32.6	7307	10/29	5.9	4.2	-16	
11/4	57.0	.03		5.07	2.24			5.36	30.1	2.6	32.7	6636	11/1	5.6	6.5	-12	11/1. Thyroxin, 10 mgm.
11/7	56.8	.03		5.10	1.87			6.16	29.3	2.3	31.6	6917	11/3	5.8	6.4	-12	
11/10	57.4	.03		6.00	1.91			6.47	32.3	4.3	36.6	7348	11/6	5.6	5.8	-14	
11/13	57.1	.05		6.01	3.00			6.35	33.4	4.0	37.4	7431	11/8	5.7	6.5	+2	11/10. Thyroxin, 10 mgm.
11/15	57.0	.04		6.72	3.02			6.40	40.4	3.0	43.4	6315	11/12	6.2	6.2	+10	11/14. Thyroxin, 10 mgm.
11/19	56.6	.02		5.66	2.67			5.37	32.5	2.9	35.4	6587	11/15	6.3	5.9	+16	
11/22	56.6	.03		5.08	2.73			4.76	46.4	2.7	49.1	6028	11/17	6.4	6.1	+21	11/18. Thyroxin, 10 mgm.
11/25	56.0	.02		5.22	2.38			5.33	39.8	4.1	43.9	6253	11/20	6.4	5.5	+12	
11/28	55.2	.04		5.79	2.35			5.88	40.8	5.1	45.9	6846	11/24	6.1	5.3		
12/1	55.0	.02		5.76	1.53			5.91	37.4	3.0	40.4	6314	11/27	7.1	6.1		
12/4	55.2	.04		6.06	1.41			6.04	32.0	4.1	36.1	7108	11/29	7.1	4.7	+6	
												12/1	7.3	5.5			
												12/3	7.5	6.5			
												12/6	6.4	6.1			

TABLE II (continued)

Date end ing per- iod	To- tal cal- orio in- take	Daily weight per num- ber	Calcium				Phosphorus				Nitrogen				Blood plasma		Basal meta- bolic rate	Treatment and remarks		
			Excretion		In- take	grams	Excretion		In- take	grams	Excretion		In- take	grams	Ca	P				
			Urino	Feces			Urino	Feces			Total									
												grams							grams	grams
12/7	27	55.2	01		0.03	1.47			5.53	28.0	4.5	32.5	44.5	7248	12/8	0.0	5.0	-8	12/8 Parathormone, 10 units daily to 1/13/1937 inclusive	
12/11	28	55.4	05	3.21	3.20	1.90	2.42	4.32	6.00	25.3	3.8	29.1	38.4	6304	12/10	7.2	4.3			
12/13	29		03	3.72	3.76	1.03	1.86	3.00	5.02	25.9	4.2	30.1	42.0	6871	12/13	7.6	4.0			
12/16	30	55.0	01	3.73	3.74	1.38	2.32	3.70	5.33	24.6	3.8	28.4	30.2	6458	12/15	7.8				
12/19	31	55.0	08	3.00	3.08	5.57	1.48	1.80	3.28	5.75	22.3	5.5	27.8	41.9	6969	12/17	7.6	0.1	-12	12/20 Thyroxin, 10 mgm
12/23	32	55.3	07	4.20	4.36	5.12	1.58	1.47	3.05	4.96	24.9	3.7	28.6	30.6	6103	12/20	7.3	6.2	-2	
12/25	33	54.0	05	3.87	3.02	4.36	2.25	1.45	3.70	3.19	31.3	3.3	34.6	33.1	6022	12/27	7.1	7.0	+7	12/27 Thyroxin, 10 mgm.
1/1	34	53.0	03	3.78	3.81	4.05	2.27	1.29	3.56	4.40	32.9	3.4	36.3	33.8	4890	12/30	8.3		+32	
1/5	35	53.6	02	3.51	3.53	3.73	1.79	1.33	3.17	4.00	27.0	3.1	30.1	30.7	4592	1/3	9.5	4.7	+33	
1/7	36	52.0	28	2.01	2.20	4.28	1.50	1.25	2.75	4.35	33.1	3.5	36.6	34.6	5429	1/5	9.2	3.7	+12	
1/10	37	51.8	11	2.12	2.23	2.94	74	1.25	1.99	2.85	25.4	2.2	27.6	21.9	4356	1/10	9.4	5.0		Teeth extracted
1/13	38	52.3	02	3.02	3.94	4.68	1.01	1.43	2.44	4.70	18.5	3.6	22.1	35.3	7441	1/13	8.5	4.4		

Third admission—1927

Date end ing per- iod	To- tal cal- orio in- take	Daily weight per num- ber	Excretion		In- take	grams	Excretion		In- take	grams	Excretion		grams			Basal meta- bolic rate	Treatment and remarks		
			Urino	Feces			Urino	Feces			Total								
					grams	grams			grams	grams		grams	grams	grams	grams			grams	grams
			11/8	39	55.3	04	61	45	72	.80	1.52	1.84	18.7	*	21.1			23.8	5177
11/11	40	55.1	04	.34	.39	82	.34	1.16	1.50	15.3	*	16.4	11.4	4238	11/11	4.5	6.9		Same diet
11/11	41	55.0	03	50	.85	.80	.56	1.36	1.67	12.6	*	13.9	12.5	4902	11/14	5.5	7.8		10 cc. 5 per cent calcium chloride solution intravenously
11/17	42	54.8	04	.22	.20	65	.22	.87	1.82	13.0	*	14.7	17.1	5521	11/16			-24	10 cc. 5 per cent calcium chloride intra-venously
															11/18				10 cc. 5 per cent calcium chloride intra-venously

\* Feces assumed to be 10 per cent of nitrogen intake

† Thyroxin was given in all instances intramuscularly

TABLE IV  
*Summary of metabolic data, with diet low in calcium      Untreated cases of tetany and normal individuals (Periods of three days' duration      Intake and output per 3 day period)*

Subject	Diagnosis	Number of periods	Calcium			Phosphorus			Nitrogen			Fasting blood levels	
			Urine	Feces	Intake	Urine	Feces	Intake	Urine	Feces	Intake	Ca	P
			grams	grams	grams	grams	grams	grams	grams	grams	grams	mgm per 100 cc.	mgm per 100 cc.
B W	Idiopathic tetany	(1-3)	0.03	0.55	0.25	0.84	0.49	1.75	18.8	2.5	19.4	4.6	7.0
K.L.*	Parathyreopriva tetany	(1-3)	0.02	0.40	0.26	0.54	0.45	1.40	8.8	1.8	14.5	4.9	5.1
DeLaB	Steatorrheic tetany on neutral diet.	(1-6)	0.01	0.71	0.25	1.44	0.71	1.40	15.8		15.9	5.4	2.8
Average of nine controls on neutral low calcium diets			0.19	0.38	0.32	1.17	0.61	2.00	24.2		28.1	9.5	3.8
Average of 13 controls on diets with uncontrolled potential acidity (8)			0.19	0.60	0.33								

\* Some parathormone was given during these periods (see Table I)

*Metabolic findings in patient with chronic steatorrhea*

The metabolic abnormalities in the patient with chronic steatorrhea were so fundamentally dissimilar from those of the patients with parathyroid tetany that they must be considered separately (see Table III). The low serum calcium and the consequent low calcium excretion in the urine (cf serum calcium below the kidney threshold) need no further comment. In periods 1-6 (Table III) the fecal calcium excretion while on a low calcium diet was perceptibly higher than that of normal individuals on a similar regime (Table IV). That this abnormality was due to lack of absorption of calcium from the gastro-intestinal tract rather than to increased excretion of calcium into the gastro-intestinal tract was well demonstrated in periods 7-11 (Table III). Here, on a higher intake of calcium, almost all of the ingested calcium appeared in the feces. This lack of absorption of calcium may apply, of course, to calcium excreted into the gastro-intestinal tract which ordinarily would be reabsorbed. In periods 29 and 30 (Table III) on a very high calcium intake there was considerable absorption of calcium and the serum calcium did rise. The abnormalities in the calcium metabolism in this case may thus be summarized as

- 1 A long continued lack of absorption of calcium,
- 2 A resulting low serum calcium due to long continued calcium privation, and
- 3 A consequent low calcium excretion in the urine because of the low serum calcium (threshold phenomenon)

There were three possible factors to account for the decreased absorption of calcium from the gastro-intestinal tract. The formation of insoluble soaps was probably the most important factor. Increased intestinal rate may have been an added factor. Finally, an increased pH of the upper intestinal tract (cf anacidity) may have played a part (9). The final proof that lack of calcium absorption was at the bottom of this disorder was shown in later studies by Bauer and Marble (9). By administering ergosterol they noted an immediate remarkable increase in calcium absorption and a later return of other abnormalities to normal.

It is now of interest to see how the phosphorus metabolism reacted to this disorder of calcium metabolism. It is apparent at once that the serum phosphorus was very low. Just as the total calcium excretion on a low calcium diet was within normal limits (periods 1-6), likewise the total phosphorus excretion was not abnormal. However, very unlike the situation in parathyroid deficiencies, there was a high partition of phosphorus in the urine as compared to the feces. Thus, in spite of the low serum phosphorus, there was a normal excretion of phosphorus in the urine. This makes one question whether phosphorus is a threshold substance at all in spite of the contention of Albright, Bauer, Clafin, and Cockrill (13) that the abnormalities in parathyroid disorders are de-

pendent on alterations in the threshold for phosphorus excretion. This point is discussed elsewhere (13). The findings in the phosphorus metabolism were, therefore

- 1 A low serum phosphorus

- 2 A normal excretion of phosphorus with a high partition in the urine as compared with the feces

A study of the total acid base balance throws further light on the metabolic abnormalities in this case. With the loss of large quantities of organic fatty acids, a large amount of base was also found in the feces, about half of which was available as alkali for the neutralization of organic acid (11). The fecal ash was markedly alkaline, probably because the ashing removed organic acids. When the fecal ash was ground with water and titrated to methyl red with normal HCl, an end point could only be approximated. During period 28, when no medication was given, it required 138 cc. normal HCl in this inaccurate titration, while 425 cc normal HCl were required in period 30 when 9 grams of  $\text{CaCl}_2$  were given daily. This large loss of base by feces naturally influenced the reaction of the absorbed part of the diet. The ingested diet was potentially neutral, the absorbed part was, in all probability, acid. This may well explain the high concentration of ammonia found in the urine (11). In agreement with such an explanation was the shrinking of urinary ammonia when sodium bicarbonate was added to the diet (periods 25-27), and the rise in ammonia excretion when ammonium chloride was ingested (periods 12-18). The fact that calcium chloride (periods 29 and 30) not only increased the fecal total base and fecal alkalinity but also the urinary ammonia was probably due to the greater absorption of the chlorine ions as was originally shown by Gamble (14).

#### *The effect of parathormone medication in hypoparathyroidism*

As a method of treating parathyroid tetany, parathormone is dramatic.<sup>2</sup> From the studies made on Cases I and II it is obvious that small doses of parathyroid extract exert a much greater effect in patients with parathyroid deficiency than in normal individuals. In our normal control cases, 100 units of parathormone daily resulted in a rise of the blood calcium level of about the same degree as 10 or 15 units did in these cases of tetany.

The effect of parathormone was more obvious on the blood calcium level than on the calcium excretion. There was only one control period in the observation upon K L, so that the effect of parathormone on the calcium excretion was not certain, but it obviously was not marked, for even after the blood calcium had risen during parathormone administration from 4.2 to 6.9 mgm per 100 cc the total calcium excretion remained

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<sup>2</sup> We wish to take this opportunity to thank Eli Lilly Co. for the generous supplies of parathormone which they gave to us for this investigation.



at the very low level of 0.37 gram in 3 days. Practically the same effect was seen in the case of B. W. He was on a high calcium diet when he was given daily injections of 10 units of parathormone. This was adequate to raise the blood calcium from 6 to 7.8 mgm, but there was no striking effect on either the fecal or urinary calcium excretion until the blood calcium had risen above 9 mgm. It is true that the high calcium intake would have hidden any minor effects upon the fecal calcium excretion, but no technical error ought to have obscured an effect on the low calcium excretion in the urine. The explanation suggested by Albright and Ellsworth (16) that the renal threshold for calcium excretion is about 8.5 mgm probably explains these findings.

In the course of our observations it has become obvious that parathormone has its most marked effects in the first few weeks of administration and that then its influence on calcium metabolism is sometimes gradually lost. Thus, its beneficial effects were strikingly observed in K. L. who lost all symptoms and signs of tetany on only 15 units (later 7 1/2 units) a day. This very satisfactory result lasted while the patient was in the hospital and on a low calcium intake. Then she was given a diet high in calcium and discharged from the hospital, but in spite of continued parathormone injections, her blood calcium gradually fell. On her re-admission to the hospital some months later, daily injections of over 100 units of parathormone would not change her low blood calcium or her total calcium excretion even though she was on an adequate calcium intake. This was not ascribable to poor parathormone because the same preparation had a marked physiological effect on other patients. We have observed such an immunity in other patients as well (17). This has also been reported by Lissner and Shepardson (18) in a striking case of tetany. In Boothby's recent studies (19) such an immunity was not observed.

#### *The effect of thyroid medication in hypoparathyroidism*

In paper III of this series (1) it was shown that the effect of thyroid on blood calcium was negligible, but that its stimulating effect on calcium excretion was of great magnitude. It was, therefore, natural to try the effect of thyroid therapy on these cases of parathyroid tetany. The resulting effect was very striking. This can best be demonstrated in the first observation on K. L. (See Table I and Figure 1.)

Prior to the administration of thyroid she had received daily intramuscular injections of 15 units of parathormone. This had raised her blood calcium from 4.2 to 6.7 mgm per 100 cc. during a period of 18 days. Without altering the parathormone dosage, thyroxin (25.0 mgm) and thyroid (2.4 grams) were administered to her during a period of two weeks. Her metabolic rate rose from minus 14 per cent to plus 22 per cent and her blood calcium rose from 6.7 mgm to 11.9 mgm per 100 cc. It was only then that the calcium excretion was influenced, increasing three-fold over

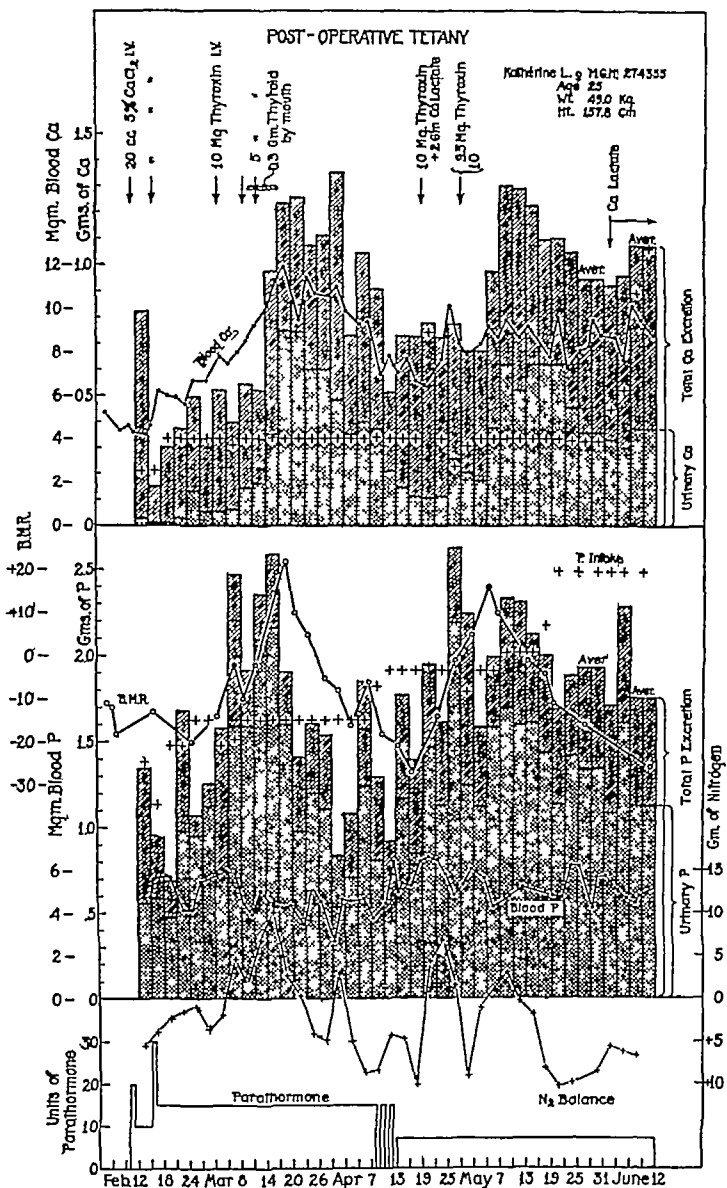


FIG 1

its value in the earlier periods. This increase came in spite of a constant low calcium intake so that it must have been derived from calcium in the bones. A large part of this increase appeared in the urine. The increased calcium in the excreta and blood persisted six weeks after the thyroid medication had been omitted. This result was re-obtained later in the same patient. The urinary phosphorus excretion (see Figure 1) was increased markedly with the giving of thyroid and long before the increased calcinuria had appeared. The serum phosphorus was little affected.

On three separate occasions in the other patient (B. W.) the blood calcium level was definitely raised by the intramuscular injection of thyroxin (see Tables, II and V). In the first observation, thyroid was given alone. After the basal metabolic rate had returned to normal, the blood calcium rose from 4.5 to 6.4 mgm per 100 cc. This elevation was maintained for almost three weeks and was accompanied by a fall in the blood phosphate level. The calcium excretion was not determined during this period. During the second observation, the patient was on a high calcium diet. The low urinary calcium output showed no acceleration as a result of thyroid, although the blood calcium value was elevated slightly more than a milligram. The level had apparently not reached that of the kidney threshold. The third administration of thyroxin to B. W. (periods 31 to 38) was superimposed upon a high calcium diet plus ten units of parathormone daily. The parathormone alone raised the blood calcium level from 6 mgm to 7.8 mgm without much effect on the blood phosphorus or urinary calcium. When thyroxin was given in addition, the blood calcium rose to 9.4 mgm and remained elevated for over two weeks and the blood phosphorus level fell for the same period. During six days the calcium excretion in the urine rose far higher than at any other time in the whole observation, although this increase did not appear for at least three days after the blood calcium level had reached its maximum. Here, then, thyroxin, superimposed on a high calcium intake plus parathormone, increased the calcium in the blood to a level which was sufficiently high to cause an increase in the urinary excretion. The effect of thyroid medication in parathyroid tetany is shown in summary form in Table V.

Administration of thyroid or thyroxin to patients suffering from hypoparathyroidism has taught us then the following facts:

1. Whereas in hyperthyroidism, in spite of a marked increase of calcium excretion, there is no definite elevation of the blood calcium, in hypoparathyroidism administration of thyroid or thyroxin results in a marked elevation of the blood calcium.
2. This elevation of the blood calcium does not lead to increased calcinuria until the blood calcium has risen beyond the threshold level.

TABLE V  
*Effect of thyroxin on average metabolic levels in cases of tetany (Intake and output per 3 day period)*

Subject	Diagnosis	Regime	Before thyroxin					After thyroxin				
			Date	Average age urinary cal cium	Average blood plasma values		Average basal metabolic rates. Variation from normal*	Date	Average age urinary cal cium	Average blood plasma values		Average basal metabolic rates. Variation from normal*
					Cal cium	Phos- phorus				Cal cium	Phos- phorus	
				grams	m gm per 100 cc.	m gm per 100 cc.	per cent	grams	m gm per 100 cc.	m gm per 100 cc.	per cent	
K L	Parathyreo- priva tetany	Low Ca diet	February 18 to March 1	06	6 2	4 5	-17	March 17 to 31	61	10 9	4 2	+5
		Low Ca diet	April 13 to 25	13	7 2	5 9	-25	May 3 to 14	54	9 0	5 0	+8
B W	Idiopathic tetany	Low Ca diet	July 17 to 30	02	4 47	7 61	-18	August 10 to 25		6 36	5 32	-7
		High Ca diet	October 12 to November 3	04	5 81	5 66	-14	November 22 to December 6	03	6 83	5 43	+15
		High Ca diet plus 10 units parathormone daily	December 10 to 23	04	7 53	5 63	-10	January 3 to 13	20†	9 20	4 44	+33

\* Aub and DuBois standards.

† Two periods only

- 3 There is an increased urinary phosphorus excretion, which begins almost immediately The serum phosphorus is little affected
- 4 Thyroid extract is a helpful adjunct in the symptomatic treatment of parathyroid tetany

In paper VIII (20) of this series, a further discussion of the mode of action of thyroid on calcium metabolism is given

*The effect of potentially acid salts on tetany*

Ammonium chloride or other potentially acid salts have been utilized by several investigators for the treatment of tetany Just as alkalosis tends to bring on tetany, acidosis tends to dispel it Because of the paucity of metabolic studies, however, we are briefly reporting the results on these patients The clinical signs of tetany disappeared and the neurological electrical reactions improved in the patient K L when she was given hydrochloric acid and ammonium chloride (periods 52 to 59) But this improvement was accompanied by only small changes in her calcium and phosphorus metabolism The blood calcium and phosphorus levels fluctuated back and forth without showing any decided change The urinary calcium excretion was approximately doubled, but, because of the initial small value, this represented only a small, actual increase The effect was slight in comparison with that of thyroid extract A similar amount of ammonium chloride in normal individuals on a low calcium diet (10) produced on the average a four-fold increase in urinary calcium, but no effect on fecal calcium (see Table VI)

In patient DeLaB, who had steatorrhea, the effect of ammonium chloride was more carefully controlled Metabolic studies of this patient showed that on a neutral low calcium test diet, she excreted approximately a normal amount of calcium but that this was almost entirely in the feces When more calcium was added to the diet (periods 7 to 11) the blood calcium rose slightly, but the urinary calcium did not rise in spite of a positive calcium balance The giving of ammonium chloride (4 grams daily in periods 12 to 15, and 6 grams daily in periods 16 to 18) then increased the fecal excretion of calcium enough to produce a negative calcium balance again, and there was a definite rise in her blood calcium from 6.0 to 7.6 mgm This elevation of blood serum calcium was associated with a marked clinical improvement All symptoms of tetany and the Chvostek and Trousseau signs disappeared only to return two days after ammonium chloride was discontinued Corresponding to the increased fecal calcium excretion there was a definite increase in the urinary phosphorus excretion Thus, whereas normally ammonium chloride causes an increased urinary excretion of calcium and phosphorus, in this case, presumably because of the low serum calcium, the increased calcium excretion was in the feces, while the increased phosphorus excretion, in spite of the low serum phosphorus level, remained in the urine

TABLE VI  
*The effect of ammonium chloride on the calcium excretion      Average values of three day periods, expressed in grams (Intake and output per 3 day period)*

Subject	Diagnosis	Number of periods	Calcium			Fasting blood levels		Medication
			Urine grams	Feces grams	Intake grams	Ca mgm. per 100 cc.	P mgm. per 100 cc.	
K. L.	Parathyreopriva tetany	46-51	0.07		2.25	4.9	6.1	15 units parathormone given daily throughout all periods
		54-55	0.10		2.25	5.2	5.4	Hydrochloric acid plus ammonium chloride—equivalent to chloride in 6.5 grams $\text{NH}_4\text{Cl}$ daily
		56-59	0.17		2.24	4.7	6.5	$\text{NH}_4\text{Cl}$ 6 grams daily
DeLaB	Steatorrheic tetany	7-11	0.01	1.23	1.52	6.1	2.5	Control diet    No medication
		12-15	0.02	1.77	1.56	6.0	3.0	Same diet plus 4 grams $\text{NH}_4\text{Cl}$ daily
		16-18	0.02	1.98	1.61	7.6	3.0	Same diet plus 6 grams $\text{NH}_4\text{Cl}$ daily
Control B E (10)	Scatica	20-21	0.31	1.08	2.12			No medication but moderate calcium diet
		15-17	1.00	1.02	2.13			$\text{NH}_4\text{Cl}$ 6 grams daily with same moderate calcium diet
		1-5 12-14	0.12 0.60	0.32 0.26	2.7 2.7			On low calcium diet $\text{NH}_4\text{Cl}$ 6 grams daily

Large doses of sodium bicarbonate added to her diet for three periods had no demonstrable effect on the calcium, phosphorus, or nitrogen excretions, nor on the blood serum levels of calcium or phosphorus

*The intravenous administration of calcium*

In early periods of study both K L and B W received occasional intravenous injections of calcium chloride. These relieved their signs of tetany temporarily, but the calcium apparently was not subsequently found in the excreta. We, therefore, studied this more carefully in B W by maintaining him on a constant, moderately low, calcium diet. After control periods were obtained, he was given repeated intravenous injections of calcium chloride in such quantities that his calcium intake was twice that of the control periods. Table II demonstrates that in this short observation all of this extra calcium was stored (as in an observation by Salvesen, Hastings and McIntosh (21)) with a reduction in phosphorus excretion in the second period approximately equivalent to the amount needed for bone deposit ( $\text{Ca} : \text{P} = 2 : 1$ ). This demonstrates a striking characteristic of parathyroid tetany, namely, the great avidity for storage of calcium and the resistance to its elimination. This retention could not be ascribed to a previous lack of calcium.

DISCUSSION

From the above rather miscellaneous assortment of data, one striking fact needs special discussion. The thyroid hormone, which raises only very slightly the serum calcium and phosphorus of otherwise normal individuals, elevates very appreciably the low serum calcium of patients with parathyroid tetany. A clarification of this phenomenon is suggested by an analysis of threshold values for excretion of calcium and phosphorus.

Albright and Ellsworth (16) point out that there is a threshold value for urinary calcium excretion, below which the calcium in the urine remains negligible. The extraordinary thing about this threshold is that it is surpassed by the normal value for serum calcium. Calcium privation, unless long continued, will not lower the serum calcium to the threshold value. There is no counterpart to this in physiology as far as we are aware. The implication is that there is another mechanism which keeps the serum calcium above this threshold, otherwise the calcium excretion in the urine would soon lower the serum calcium to the threshold value. This other mechanism may well be the parathyroid hormone.

Phosphorus is thought by Albright, Bauer, Clafin and Cockrill (13) to be a threshold substance. The normal value for serum phosphorus is thought by them to represent approximately the threshold value. The high serum phosphorus level of hypoparathyroidism and the low serum phosphorus level of hyperparathyroidism are thought by them to represent not levels above and below the threshold respectively, but changes in the threshold values.

Now the thyroid hormone, regardless of the exact mechanism, mobilizes large amounts of calcium and phosphorus from the bones into the blood stream and hence into the excretory channels. Thus, one would expect it in the normal state to cause a slight rise of the blood levels of both calcium and phosphorus and, because both thresholds would then be exceeded, to produce an immediate excretion of both. This is just what occurs. In hypoparathyroidism, however, the calcium on arriving in the blood stream still is below the threshold value and would not be immediately excreted. This is not true of the phosphorus. There would, therefore, be a tendency for the serum calcium to rise without any appreciable alteration in the serum phosphorus. This is just what occurs.

#### CONCLUSIONS

- I Previously noted alterations in the calcium and phosphorus metabolism in parathyroid tetany are confirmed, viz ,
  - a* A low serum calcium level
  - b* A high serum phosphorus level
  - c* A low urinary calcium excretion with an unaltered fecal calcium excretion
  - d* A low urinary phosphorus excretion with an unaltered fecal phosphorus excretion,—hence a low partition of phosphorus in the urine as compared with the feces
- II The alterations in the calcium and phosphorus metabolism in the tetany associated with chronic steatorrhea have the following points of similarity with parathyroid tetany
  - a* A low serum calcium level, and
  - b* A low urinary calcium excretion,  
but the following points of dissimilarity
    - a* A low serum phosphorus level,
    - b* A high fecal calcium excretion, and
    - c* A high urinary phosphorus excretion,—hence a high partition of phosphorus in the urine as compared with the feces
- III In our patient with steatorrhea all the disordered calcium and phosphorus metabolism was dependent on a decreased calcium absorption from the gastro-intestinal tract. This was probably due to three factors
  - a* The formation of calcium soaps,
  - b* The increased intestinal rate, and
  - c* The decreased acidity of the gastric contents
- IV
  - a* A given dose of parathyroid extract is more efficacious the greater the degree of hypoparathyroidism.
  - b* Some patients with long continued injections of the present preparation of parathyroid extract become refractive to the drug



- V Thyroid medication has the following effects in hypoparathyroidism
- a* To raise the serum calcium markedly
  - b* To increase the calcium excretion in the urine, but only after the serum calcium has surpassed the threshold value
  - c* To increase the phosphorus excretion in the urine, without any decided change in the serum phosphorus level
  - d* To alleviate the symptoms of tetany
- VI The presence of a threshold for calcium excretion in the urine is confirmed This threshold is below the normal level of serum calcium In tetany any agent such as thyroid or parathormone which increases the level of serum calcium will not increase the urinary calcium excretion until the threshold has been passed The excretion of calcium into the gastro-intestinal tract, inasmuch as it is not decreased with the low serum calcium of parathyroid tetany, is probably not a threshold phenomenon
- VII The question of a threshold level for phosphorus excretion in the urine cannot be decided on the data presented Two pertinent facts appear
- a* In hypoparathyroidism with a high blood phosphorus the urinary phosphorus excretion is reduced
  - b* In the tetany of chronic steatorrhea with a very low blood phosphorus the urinary phosphorus excretion is normal
- VIII In explanation of the phenomenon that thyroid medication raises the serum calcium of patients with hypoparathyroidism appreciably while its effect on the serum of normal patients is almost negligible, the following hypothesis is suggested in hypoparathyroidism the calcium on being taken from the bones by the thyroid hormone finds itself in the blood below rather than above the threshold for excretion and hence is not immediately excreted

## APPENDIX

### CASE HISTORIES

*Case I* Miss K L, M G H no 274355, a white, unmarried woman, twenty-five years of age, was admitted to the Hospital, January 28, 1926, and was discharged January 27, 1927 The discharge diagnosis was Post-operative parathyroid and thyroid deficiency

*History of present illness* Miss K L was first a patient in this Hospital in 1917 At that time she was suffering from mild hyperthyroidism for which x-ray treatment was advised She refused treatment and consulted another physician He performed a subtotal thyroidectomy Six months later, because of the return of symptoms of hyperthyroidism, another subtotal thyroidectomy was performed She stated she had been hoarse since this last operation Two months later she first noticed difficulty in breathing Wheezing was always present but was made worse by cold and exertion Besides this asthmatic-like breathing, she had frequent attacks of carpopedal spasms

during which times she was unable to talk. Prior to admission to the Hospital these attacks of tetany (laryngeal spasm) were so severe that she was unable to breathe for several minutes at a time. Following her second operation, she first noticed dimness of vision. This rapidly increased, finally necessitating the removal of bilateral cataracts.

*Physical examination* She was a well developed, well nourished young woman with slightly labored breathing and a hoarse voice. Her skin was somewhat dry and coarse with scaling over the shins. Her hair was dry and coarse. The eyes, ears, nose, and throat showed no abnormalities. The heart was not enlarged. No murmurs were heard. Blood pressure was 100/70. Examination of the chest revealed slight dullness over either back. The breath sounds were prolonged accompanied by expiratory wheezes. Occasional sibilant râles were heard throughout the chest. The abdominal examination was negative except for voluntary muscle spasm. There was slight brawny edema of the ankles. The nails were coarse and very brittle. The Chvostek and Trousseau signs were positive. The reflexes were all hyperactive.

*Laboratory findings* Five urine examinations revealed no abnormality. Blood examination showed a hemoglobin of 75 per cent, erythrocytes 5,656,000, leucocytes 7,900. The differential leucocyte count was normal and the smear was negative except for marked achromia. The phenolsulphonephthalein test was 60 per cent. The Wassermann test was negative. The nonprotein nitrogen was 28 mgm per 100 cc. The serum calcium was 5.2 mgm per 100 cc and the serum phosphorus was 5.4 mgm per 100 cc. Basal metabolism was -11 per cent. A ray examination of the skeletal system showed no deviation from the normal.

*Progress notes* During her stay in the Hospital, she had frequent attacks of severe tetany with marked laryngismus. These gradually disappeared when parathormone and thyroid medication was given. However, she eventually became so refractory to parathyroid extract that 100 units a day did not keep her free from the signs and symptoms of tetany.

On January 23, 1927, she complained of toothache accompanied by a temperature of 101 to 102° F. X ray examination of her teeth showed several apical abscesses. Extraction of these teeth was advised, hoping that the elimination of these foci of infection might possibly benefit her. During the administration of ethylene anesthesia she developed severe laryngismus. This was not relieved by small amounts of ether, adrenalin, or 5 per cent calcium chloride intravenously. Finally a tracheotomy was performed. This procedure relieved her laryngismus but she was then in a shock like condition with a low blood pressure. All subsequent treatment was without effect and she died in the surgical amphitheatre.

*Autopsy* No 5119 January 27, 1927, by Dr. Tracy Mallory. Anatomical diagnoses: Parathyroid and thyroid deficiency, persistent thymus and focal necrosis of the liver. Only a small remnant of thyroid tissue, measuring 22 × 9 × 9 mm remained. This was firmly adherent to the cricoid cartilage. It contained considerable fibrous tissue in bands which separated the islands of parenchyma. The thymus was large, having roughly the shape of the numeral 8 with an isthmus, small upper and large lower poles. From top to bottom it measured 11 cm and from side to side 6.5 cm, but it was not more than 3.5 mm in thickness. It weighed 21 grams. In the fibrous tissue overlying the trachea several small, pinkish masses averaging 2 mm in diameter were found. Microscopic examinations were not remarkable except as follows: Thymus—Infantile type, well differentiated cortex and medulla. Thyroid—

The remnant of the thyroid showed a marked increase in fibrous tissue, irregular in distribution. A few acini were greatly dilated, however, the majority were small, with a tendency to hyperplasia of the epithelium. No remnants of parathyroid tissue were found in the surrounding fibrous tissue. Neck tissue—Examination of the eight small glands removed from the neck showed no parathyroid tissue. The liver showed small areas of focal necrosis and invasion with polymorphonuclear cells.

*Case II* Mr B W, M G H no 277407, a white, married Jewish tailor, 52 years old, was first admitted to the Hospital on July 10, 1926, and discharged August 30, 1926. He was re-admitted October 11, 1926, and discharged January 20, 1927. The patient had felt well until ten weeks before entrance to the Hospital. At this time he first noticed a general feeling of uneasiness with mild, irregular muscle spasms in his hands, forearms, and legs. A week later he fell in the street because of marked contractions of arms and legs. He did not lose consciousness, felt no pain, but his extremities seemed anesthetic. The attack lasted three or four hours, and was followed by repeated attacks of a shaking sensation of his muscles but without evidence of muscle contractions. He had a second severe attack three weeks prior to his first entrance. Otherwise he had only local muscle spasms of the arms and legs which recurred every few minutes. Five weeks before entrance to this Hospital he noticed his eyesight was failing, necessitating his giving up his position as a tailor. Seven days prior to his hospital entrance, he developed severe pain in both shoulders on motion.

*Physical examination* The physical examination disclosed nothing abnormal save a few very carious and infected teeth, evidence of a bilateral subdeltoid bursitis, and very markedly positive Chvostek and Trousseau signs.

*Laboratory findings* The routine urine and blood examinations were normal. The Wassermann test was negative. The serum calcium was 5.1 mgm per 100 cc and the serum phosphorus was 7.3 mgm per 100 cc. The blood CO<sub>2</sub> combining power was 71.8 volumes per cent. The basal metabolism tests were -15 to -20 per cent. The nonprotein nitrogen was 38 mgm per 100 cc. Gastric analysis showed acid values within normal limits. The electrical reactions were typical of those found in parathyroid tetany.

*Case III* Mrs DeLaB, M G H no 290165, was a white, married stenographer, 27 years of age. She considered herself well until five years prior to her entrance into the Hospital. At that time she weighed 123 pounds. She gradually lost in strength and energy. At the time she entered the Hospital she weighed 93 pounds. Four years previous she had developed "mild indigestion" associated with epigastric distress coming on about an hour after meals and lasting several hours. This was sometimes accompanied by nausea and rarely by vomiting. Large meals accentuated all of the above symptoms. She had suffered from alternating diarrhea and constipation all her life. For the past four years she had noticed twitchings of the face, areas of paresthesia over scalp and back, and frequent attacks of carpopedal spasm.

*Physical examination* Physical examination was completely negative except for areas of paresthesia and markedly positive Trousseau and Chvostek signs.

*Laboratory examination* Routine blood and urine examinations were negative. Phenolsulphonephthalein test was 60 per cent. Basal metabolism test was minus 4 per cent. The electrical reactions were characteristic of tetany. X-ray examination of the bones were negative except for slight decalcification. Gastric analysis revealed an anacidity. The feces contained much excess fat.

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# STUDIES OF CALCIUM AND PHOSPHORUS METABOLISM

## XV IN VARIOUS METABOLIC AND BONE DISEASES

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The bones may well be looked upon as a storehouse for calcium and phosphorus, readily available for the body needs for fixed base. The demands upon this supply can readily be determined if a carefully controlled diet inadequate in calcium is administered. Under such conditions the normal excretion of calcium remains fairly constant, and a comparison with disease states may readily be undertaken.

For several years this laboratory has systematically studied the calcium and phosphorus exchange in numerous pathological conditions. In the course of these studies various diseases have been investigated and are to be published (1, 2, 3).

We have also had the opportunity to investigate many interesting diseases in which a disturbance of bone metabolism might well be expected. Some of these isolated cases are here brought together and this paper, therefore, demonstrates the metabolic need of calcium and phosphorus in various bone abnormalities as well as in gout and chronic hepatitis with jaundice.

### EXPERIMENTAL METHODS

The patients were, with one exception, studied in the metabolism ward at the Massachusetts General Hospital. The careful management and routine used there for the preparation of accurate diets and for the collections of urine and feces has already been fully described (4). The periods used here were of three days' duration, and the feces were divided by the appearance of carmine. The methods of analysis for calcium and phosphorus were those of Fiske (5) and the nitrogen determinations were made by Kjeldahl method. In order to determine the endogenous need for calcium the patients received our usual neutral diet, which is inadequate only in calcium. In nine normal control subjects on similar neutral diets, the excretion of calcium averaged 186 mgm. in the urine and 386 mgm. in the feces per period.

### A DISEASES AFFECTING THE SKELETON

#### *Secondary carcinoma involving bone*

In the course of studies on the effect of lead therapy upon cancer growth, observations were also made upon the calcium and phosphorus

metabolism of patients with secondary breast carcinoma involving the spine, pelvis, and other bones. The calcium excretion of most of these patients fell within the normal range. In one patient, however, whose lesion in the pelvis was progressing, there was a very high urinary calcium excretion.

Case I, Mrs. B., age 47, weighing 48 kilos, had the left breast removed in 1925, and the right in June, 1927, both breasts being infiltrated with carcinoma. For approximately six months before her admission to the hospital, she had suffered from stiffness and soreness in the back and right shoulder and, for some weeks immediately preceding admission, with more severe pain low in the back. She was fairly comfortable when lying still but was afraid to move. X-ray examinations revealed metastases in the spine, ribs, and pelvis, with pathological fracture of a vertebra, and some weeks later showed that the process in the pelvis had rapidly advanced. The values for calcium determinations are presented in Table I.

TABLE I

*Secondary carcinoma involving bone  
(Intake and output in 3 day periods)*

Subject and diet	Period	Calcium				Phosphorus		Serum		
		Urine	Feces	In- take	Balance	Urine	In take	Ca	P	Pro- tein
	(3-days)	grams	grams	grams	grams	grams	grams	mgm per 100 cc	mgm per 100 cc	grams per 100 cc
Subject B M—Case I Low Ca diet	1	92	58	25	-1 25	1 02	1 50	10 7	5 1	6 8
	2	1 11	66	25	-1 52	1 31	1 50			
	3	1 33	29	26	-1 36	1 55	1 50			
	4*		37	26		1 62	1 50			
	5*	1 19	25	28	-1 16	1 59	1 50			
	6*	92	40	35	- 97	1 61	1 47			
	7*	48	39	32	- 55	1 09	1 39			
	8	62	22	38	- 46	95	1 47			
High Ca diet	9*	77	1 06	2 83	+1 00	1 43	3 43	10 5	3 9	
	10*	69	1 05	3 18	+1 44	1 38	3 43			
	11	49	1 15	3 19	+1 55	1 59				
Subject I N—Case II Low Ca diet	1	33	27	34	- 26		2 04	9 3	3 6	7 3
	2	35	36	33	- 38					
	3*	41	29	31	- 39					
	4*	59	26	32	- 53					

\* 60 mgm of lead injected intravenously in the form of colloidal lead phosphate

In the initial periods on a constant, potentially neutral diet low in calcium, the urinary excretion of calcium was very high, the calcium of the feces being within normal limits. There was a large negative calcium

balance While she remained on the same diet the urinary calcium excretion fell to a level within the normal range Then she was given a high calcium diet on which the calcium of the urine remained at the relatively low level while the fecal calcium increased greatly and there was a positive balance The serum calcium did not change

It seems reasonable to associate the excretion of large amounts of calcium in the urine with the abnormal liberation of calcium about the enlarging, destructive lesion in the pelvis The periodicity of growth of malignant disease in bone is generally recognized As the activity decreases temporarily the flow of calcium from the bone into the excretory channels would naturally diminish and the calcium of the urine fall toward normal amounts It is interesting that in this case the subsequent ingestion of a diet fairly high in calcium, which gave rise to a large positive calcium balance, had little effect on the amount of calcium in the urine or on the serum calcium level The same thing has been found in other cases

We do not attribute the change in calcium excretion to the effect of injected colloidal lead, as we have had no evidence in other cases to indicate that lead given under the conditions of our work had any specific effect on the tumor growth or on calcium metabolism

Case II, Mrs. L. N. age 33, weighing 62 kilos, suffered from extensive metastatic carcinoma involving the skull, spine, pelvis, and humerus The data for calcium and phosphorus excretion in her case are also given in Table I In spite of the extensive malignant disease the calcium excretion during the period of observation was within the normal range A very marked clinical improvement after lead and x-ray therapy with recalcification of tumor masses is interesting though of doubtful significance.

### *Myeloma*

It was interesting to compare the calcium metabolism in a patient suffering from multiple myeloma with that of cases of breast carcinoma with metastases in the bones Case III (Mrs. M.), age 27, weighing 44 kilos, had suffered for approximately two years with darting pains in various bones, which followed sudden movements She was comfortable when resting, but weak and pale X-ray examinations demonstrated a diffuse and destructive process involving the spine, ribs, scapulae, humeri, pelvis, and heads of the femora Some of the areas of destruction were quite large with sharply defined margins The 9th dorsal vertebra was mushroomed The appearance suggested to Dr. George Holmes either myeloma or metastatic carcinoma Large amounts of Bence Jones protein were found in the urine The pathological diagnosis of myeloma was made from sections of bone removed at biopsy Results are given in Table II





The excretion of calcium and phosphorus in both urine and feces was within the normal range for adult patients on a low calcium diet (6). It is notable that in spite of wide-spread involvement of bone there was no increased excretion of calcium. Due to a very low urinary calcium excretion, the negative balance was actually less than that of the average normal adult. From the clinical point of view also the disease appeared to be relatively stationary.

#### *Focal osteitis fibrosa*

Case IV<sup>1</sup> (Miss R.), aged 34, weight 53 kilos, had been well up to three years ago, when a fracture of the right femur occurred at the site of a large cyst. At open operation the cyst wall was curetted, following which firm bony union occurred. The report of histological examination of the curettings described "a cell rich fibrous tissue containing bone trabeculae with areas of hemorrhage and blood pigment. The histological appearances are those of osteitis fibrosa." In 1928 she began to suffer severe shooting pain in the right ankle. X-ray examination revealed a large cyst in the lower end of the right tibia and another in the right pubis, but the rest of the skeleton appeared to be normal. The cyst in the tibia was opened and its wall curetted. The pathological examination revealed a similar picture to that previously reported.

Before operation she was given a low calcium diet for nine days. In Table II are presented results of the calcium and phosphorus determinations.

The excretion of calcium and phosphorus was within normal limits and the blood serum values were normal. On a higher calcium intake part of this calcium was retained as in normal individuals. The normal calcium metabolism in this case of focal osteitis fibrosa is in striking contrast with that of cases of generalized "osteitis fibrosa cystica" (hyperparathyroidism) (1) in which the serum calcium is high and calcium excretion greatly increased. There is an equally great contrast in the clinical picture of the two conditions.

#### *Fragilitas ossium*

Case V (May T.), age 14, weighing 25 kilos, had a typical history of fragilitas ossium. One femur had been broken at birth and x ray examination the next day also showed healed fractures of both femora. There had been repeated fractures from the slightest trauma in early years. When she was 4 years old attention was drawn to the fact that her trunk was unusually short and this has become progressively more marked since that time. Her diet, as described, had been adequate in both calcium and vitamin D. On admission she presented a very unusual appearance.

<sup>1</sup>After this article had gone to press similar findings were reported by Donald Hunter, Hyperparathyroidism Generalized Osteitis Fibrosa Brit. J. Surg., 1931-32, 212, 203.

with great deformity due to the shortness of the trunk, which exhibited a broad kyphotic curve and a gross scoliosis. The head was large and the upper extremities appeared normal, but there was extreme deformity of the lower limbs which had suffered repeated fractures. Apart from the skeletal changes no abnormalities were found.

On x-ray examination all the bones showed increased radiability with coarse irregular trabeculation and thin cortex. The findings, according to Dr. George Holmes, were those of osteomalacia.

The calcium excretion on a low calcium diet was within the limits of normal, although the urinary calcium was probably a little higher than in most children of her age. Serum calcium was normal and serum phosphorus slightly above the usual level for children. More than three years since this observation her bones remained unchanged, as judged by x-ray photographs, in spite of ample calcium and vitamins in her diet.

#### *Osteosclerosis (marble bone disease)*

Case VI (Mrs. H. B.), P. B. B. H. No. 67748, age 41, weighing 63.7 kilos, complained that for eight months she had dull pain in the back of the neck and shoulders with limitation of motion. Her previous history was interesting. A very severe diabetes started ten years ago with diabetic coma relieved by insulin eight years ago. The removal of an adenoma of the thyroid four years ago greatly improved the diabetes and also relieved attacks of cardiac palpitation. X-rays of nearly the whole skeleton disclosed a marked osteosclerosis which had not obviously increased in the past four years. All the bones except the ribs were unusually dense and rather structureless in appearance but with very thick cortices. There were no areas of decreased density as seen in Paget's disease. Four other members of the immediate family had similar findings by x-ray examination.

TABLE III  
*Osteosclerosis Case VI (Mrs. H. B.)*  
(Intake and output in 3-day periods)

Period	Calcium				
	Excretion			Intake	Balance
	Urine	Feces	Total		
(3 days)	grams	grams	grams	grams	grams
2	19	06	25	20	- 05
3	20	13	33	20	- 13
4	20	19	39	20	- 19
5	15	18	33	20	- 13

#### *Blood serum values*

Calcium—8 mgm per 100 cc., 10 mgm per 100 cc., Phosphorus—3.2 mgm per 100 cc., 3.2 mgm per 100 cc., Total protein—8.0 grams per 100 cc., Albumin—5.6 grams per 100 cc., Globulin—2.4 grams per 100 cc.

Extensive laboratory studies disclosed no variation from the normal, except a slight elevation of sugar in the blood. A study of the calcium exchange is shown in Table III. The findings are not abnormal except possibly a diminution in fecal calcium excretion. This is of less significance considering her basal metabolic rate of - 19 per cent. Therefore, further studies of phosphorus and nitrogen balance were not made in this case.

*Osteochondritis deformans juvenilis (Legg's disease)*

Case VII (Robert W<sup>\*</sup>), M. G. H. No. 279602, age 10, weighing 25.5 kilos, was an adopted child. Birth was two months premature. His childhood had been characterized by a limping gait and an avoidance of such activity as walking upstairs. These signs had become progressively more marked. He had lived upon a vegetarian diet all his life. Physical examination disclosed a short, stocky, well nourished boy with nothing abnormal to be found except in his skeleton. There was normal, free movement of all his joints. The right leg was shorter and smaller than the left with slight atrophy of the muscles. The x-rays, taken at the Boston Children's Hospital, included all the bones in the body. They showed a wide spread disturbance practically limited to the epiphyses. This disturbance was characterized by delayed development, mushrooming of the weight-bearing epiphyses, and increased density of the bone along the epiphyseal margins. In some areas, particularly the spine and pelvis, this had resulted in absence of parts of the bone. The diagnosis of generalized osteochondritis deformans was made by all the many physicians who saw him.

Prolonged metabolic studies were undertaken with both a low and a high calcium diet, with the addition of parathormone and cod liver oil. These results are difficult to interpret because there are practically no data of the normal excretion of calcium at this age period. When compared to other growing children the urinary calcium excretion is slightly elevated. This is particularly true, for his diet was essentially neutral throughout the whole observation. In spite of the abnormality of most of his epiphyses he had no difficulty in storing calcium at a rapid rate.

The abnormality of this boy's metabolism was in the phosphorus excretion. This was distinctly above the theoretical value to be derived from the calcium and nitrogen balances (see Paper IV (7)) for the first nine periods. Thereafter, more phosphorus was retained than could be theoretically explained. This change appeared before cod liver oil was given and followed the distinct elevation in plasma calcium produced by parathormone.

The nitrogen excretion and the blood findings are not abnormal for a growing boy. The results of these studies are given in Table IV and Figures 1 and 2.

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\* Referred by Dr. Robert Osgood.

TABLE IV  
*Osteochondritis deformans juvenilis Case VII (Robert W)*  
*Intake and output in 3 day periods*

Dates ending period	Period number	Calcium				Phosphorus				Nitrogen		Total caloric intake	Date	Blood plasma		Basal metabolic rate	Treatment and remarks	
		Excretion			In take	Excretion			In take	Urine Excretion	grams			mgm per 100 cc	Ca			P
		Urine	Feces	Total		Urine	Feces	Total										
10/29	1	0.29	0.22	0.51	0.21	1.35	0.17	1.62	1.56	11.6	20.6	4,431	10/29	10.1	3.9	-9*	Started on low Ca diet	
11/1	2	0.29	0.11	0.41	0.25	1.86	0.27	2.13	1.57	14.2	20.6	4,740						
11/7	3	0.31	0.12	0.43	0.21	1.81	0.34	2.19	1.54	13.8	19.7	4,526						
11/10	4	0.33	0.30	0.63	—	1.88	0.30	2.18	1.96	11.4	17.9	4,167	11/9	10.5	5.0		Started on diet of Ca 750 mgm	
11/13	5	0.16	1.05	1.51	2.25	2.13	0.83	2.96	3.05	14.2	24.4	6,066					Ca tolerance test	
11/16	6	0.12	0.71	1.11	2.25	2.12	0.68	2.80	3.05	13.9	24.4	6,111					Started on parathormone 10 minims daily	
11/19	7	0.56	0.92	1.47	2.25	2.56	0.82	3.38	3.05	14.6	24.4	6,066	11/17	9.9	4.1		Parathormone stopped	
11/22	8	0.73	0.53	1.25	2.25	2.86	0.68	3.54	3.05	15.4	24.4	6,066	11/22	11.9	5.7			
11/25	9	—	0.90	—	2.25	—	0.78	—	3.05	—	24.4	6,066						
11/28	10	0.53	0.17	1.00	2.25	1.36	0.53	1.88	3.17	21.1	26.8	6,361						
12/1	11	0.19	0.67	1.16	2.25	1.25	0.74	1.99	3.05	16.5	24.4	6,066					Started on cod liver oil 5u tid	
12/4	12	0.57	0.56	1.12	2.25	1.31	0.62	1.95	3.05	15.0	24.4	6,066						
12/7	13	0.51	0.57	1.11	2.25	1.21	0.75	1.99	3.05	14.4	24.4	6,066						
12/10	14	0.41	0.50	0.91	2.25	0.93	0.69	1.62	3.07	11.2	24.4	6,066					Started on ultra-violet lamp treatment	
12/13	15				2.25	1.39		3.07		15.6	24.4	6,066	12/13	10.9	4.7			

\* From Children's Hospital record

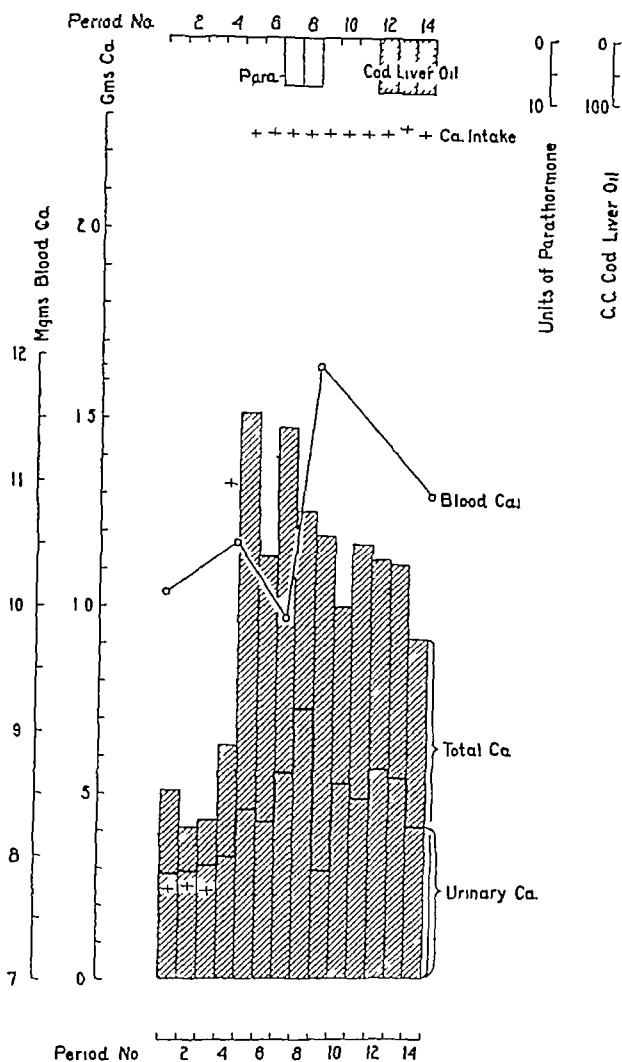


FIG 1 CALCIUM METABOLISM IN CASE VII

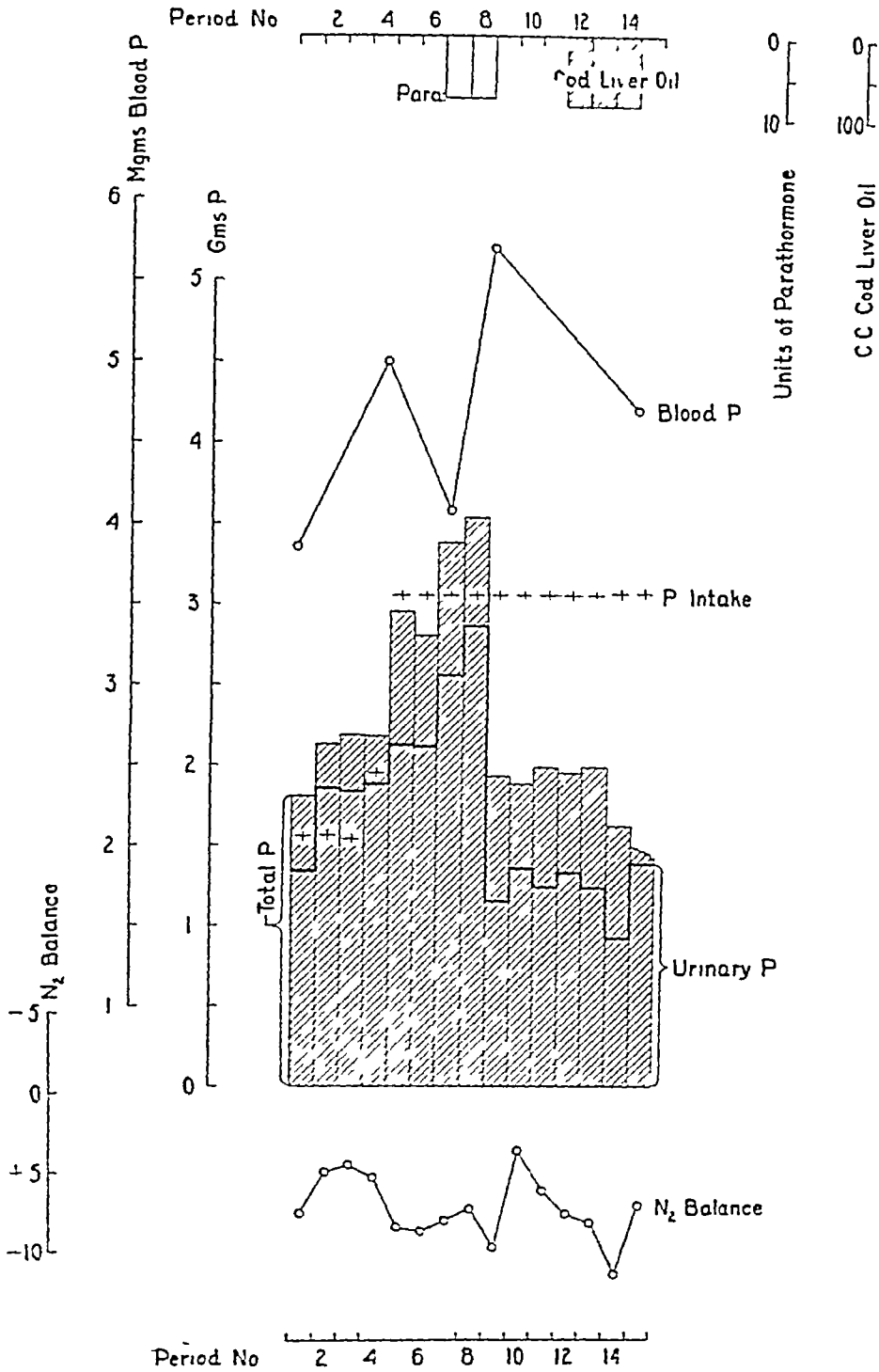


FIG 2 PHOSPHORUS METABOLISM IN CASE VII

## B OTHER METABOLIC DISEASES

*Gout*

Case VIII (H H), age 22, weighing 49 kilos, had suffered from recurring attacks of gout for five years. Many joints were involved and finally he became a complete invalid. There were numerous tophi in ears, fingers, and toes. Both olecranon bursae were involved. The metacarpophalangeal and interphalangeal joints of both hands were swollen and the interosseal muscles were atrophic. There was great thickening of the common tendon sheath of the right palm. The prepatellar bursa was similarly affected. There was very marked swelling of the joints of the ankles and feet on both sides with preternatural mobility of the great toes. From an open sinus on the right great toe a chalky material made up of crystals of sodium biurate could be easily expressed. X-ray plates showed complete destruction of the articular surfaces of both first metatarsophalangeal joints with extensive destruction of the adjoining bones. There was a varying degree of destruction of the joints of the hands. The blood uric acid was 10.3 mgm on one occasion and 12.4 mgm on another. Results of studies of calcium and phosphorus metabolism are given in Table V.

TABLE V  
*Gout Case VIII (H H)*  
(Intake and output in 3-day periods)

Period	Calcium				Phosphorus				Nitrogen		Serum	
	Urine	Feces	Intake	Balance	Urine	Feces	Intake	Balance	Urine	Intake	Ca	Protein
	GRAMS	GRAMS	GRAMS	GRAMS	GRAMS	GRAMS	GRAMS	GRAMS	GRAMS	GRAMS	mgm per 100 cc.	GRAMS per 100 cc.
1	09	34	29	- 14	1.21	52	1.31	- 42	22.4	25.0	9.7	7.43
2	16	56	29	- 43	1.23	71	1.82	- 12	19.6	25.0		
3	06	37	17	- 26	1.10	44	.97	- 57	16.0	12.6		
4	10	1.03	1.54	+ 41	.98	1.14	2.03	- .09	17.8	18.9		
5	05	1.15	1.63	+ 43	.98	1.43	2.68	+ 27	13.3	29.6		
6	09	.85	1.66	+ 73	1.10	1.07	2.45	28	19.0	29.8		

On a low calcium diet the calcium metabolism was essentially normal. When the calcium intake was increased five fold there was, as in Cases I and IX, no increase in the urinary calcium, although the fecal output increased considerably. On this diet, calcium was retained in the body.

*Chronic hepatitis with jaundice*

It has been shown that in experimental obstructive jaundice the serum calcium may be low (8). In animals with a bile fistula Whipple (9) noted that the bones became thin and spontaneous fractures occurred. It



seemed worth while to study the calcium and phosphorus metabolism of a case of chronic hepatitis with jaundice

Case IX (P N), a patient of Dr Chester Jones, age 35, weighing 51 kilos, had suffered from chronic hepatitis with jaundice (biliary cirrhosis) for 1 1/2 years. The jaundice ran an undulant course, gradually becoming worse. She was moderately jaundiced, thin, and looked weak and ill. The liver was grossly enlarged, extending as far as the umbilicus, and the spleen was readily palpable. There was no ascites or edema. Serum bilirubin was 7 to 8 mgm per 100 cc, bile pigments were present in the urine, and serum protein and blood nonprotein nitrogen were normal. Results are presented in Table VI.

TABLE VI  
*Chronic hepatitis with jaundice Case IX (P N)*  
(Intake and output in 3-day periods)

Period i	Calcium				Phosphorus				Serum	
	Urine	Feces	Intake	Balance	Urine	Feces	Intake	Balance	Ca	P
(3 days)	grams	grams	grams	grams	grams	grams	grams	grams	mgm per 100 cc	mgm per 100 cc
1	17	51	39	- 29	1 07	57	1 79	15	8 5	3 3
2	10	66	37	- 39	1 20	54	1 88	14	9 8	3 5
3	12	3 95	3 41	- 66	1 19	1 01	2 93	73		
4	14	2 42	3 15	+ 59	1 52	1 17	3 07	38	8 9	3 6

The serum calcium on two of three occasions was slightly below normal. Excretion in stool and urine was within normal limits. The change to a high calcium diet, as in Case I, had no appreciable effect on the urine calcium but was associated with an increase in the fecal calcium and phosphorus.

### *Osteomalacia*

Since this paper was written, a further type of bone abnormality has been studied—osteomalacia due to a dietary deficiency in calcium.

Mrs L H, P B B H No 39821 (Medical), aged 64, weighing 63 5 kilos, had largely avoided milk and green vegetables in her diet. Six years ago she had symptoms suggesting arthritis of the spine. Five years ago she had a slight fall and fractured two thoracic vertebrae. Convalescence has been very slow and she is still wearing a supporting cast. Except for her bones, her physical examination was not abnormal for a woman of her age. X-rays of her whole skeleton disclosed marked generalized decalcification. The thoracic and lumbar spine also showed collapse of the central portions of the bodies and apparent marked expansion of the intervertebral discs. Several of the vertebrae in the mid-thoracic region showed marked collapse suggesting spontaneous fractures.

Laboratory data. The Wassermann was negative and the urine, feces, basal metabolic rate, blood urea nitrogen, blood sugar, and blood morphology were all within normal limits. There was no excess of fat or fatty acids in the feces. The blood serum calcium level was 9 8 mgm and 10 6 mgm per 100 cc. The serum phosphorus was found to be 4 0 and 4 8 mgm per 100 cc.

*Mrs L H aged 64, white, female*  
*(Intake and output per 3-day period)*

Calcium					
Period	Excretion			Intake	Balance
	Urine	Feces	Total		
	grams	grams	grams	grams	grams
1	29	50	79	.26	— .53
2	40	40	80	28	— 52
3	1 11	2 16	3 27	5.56	2 29
4	1 03	2 55	3 58	6 00	2 42

These data disclose normal blood values and a normal calcium excretion. When a diet with a moderate amount of calcium was given to her, a large proportion of this added calcium was retained. It is interesting that the organism can maintain normal blood levels and normal calcium excretion even with such marked decalcification of the bones.

#### DISCUSSION

The changes in deposition or removal of bone salts probably occur so slowly that the variation from normal cannot be observed by our methods in relatively short observations. The observations here reported do show, however, that there is nothing grossly abnormal in the blood level or in the excretion of calcium or phosphorus. The reason for this may well lie in the large amount of calcium readily available for utilization in times of need, such as occurs on our diet inadequate in calcium. This storehouse in the trabeculae, described by Bauer, Aub and Albright (10), apparently exists in all the diseases here studied, and is available for the liberation or storage of calcium. The method determining calcium exchange, therefore, does not necessarily disclose abnormalities which might be occurring in one part of the skeleton, as there is this compensatory mechanism still present in other bones. Calcium and phosphorus may well be liberated from one part and deposited in another portion of bone. It is, therefore, not surprising that in this series of patients suffering from various types of bone diseases or chronic jaundice the variations from normal in calcium and phosphorus excretions are relatively slight.

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## INTRAVENOUS INJECTIONS

### A STUDY OF THE COMPOSITION OF THE BLOOD DURING CONTINUOUS TRAUMA TO THE INTESTINES WHEN NO FLUID IS INJECTED AND WHEN FLUID IS INJECTED CONTINUOUSLY

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It is well known that the volume of circulating blood is reduced in shock. If the reduction is so great that the loss cannot be compensated for by further vasoconstriction, a decline in the arterial blood pressure results. There are two methods by which the blood pressure may be raised. First, drugs that cause the arterioles to constrict may be injected. The pressure in the larger arteries rises but the effect on the blood supply to an organ may be decreased by the constriction of the arterioles in that organ. Since an increase in the supply of blood to the tissues and not a high blood pressure is the primary object in view, the use of vasoconstrictor drugs is usually unsatisfactory. Second, fluids may be injected intravenously in an effort to increase both the blood volume and the blood pressure.

Solutions of widely varied physical and chemical characteristics have been used in the treatment of shock. The results obtained by the intravenous administration of fluids, other than whole blood or possibly gum acacia, have not been encouraging. Cannon (1) states, "All the evidence, both clinical and experimental, indicates that the intravenous injection of normal salt solution or Ringer's solution has only a temporary effect. The injected fluid promptly passes into the tissue spaces and within a brief period the pressure is as low as before, if not lower." The rapid loss of solutions of crystalloids from the blood stream explains the transitory beneficial effects of the injection but does not explain why the condition of the patient should often be worse shortly after the administration of fluid than it was before. The experiments described in this paper were undertaken for the purpose of finding an explanation for this observation. The studies have been extended to a comparison of the effects of the administration of various fluids that are commonly used in the treatment of shock.

The studies consisted in the main of repeated determinations of the amount and composition of the plasma in the blood stream under the various conditions. Alterations in the content in protein of the blood

serum will be particularly emphasized. Trauma to the intestines was chosen as the method of producing shock because the trauma could be continuous and fluid could be recovered from the peritoneal cavity as frequently as desired for study.

### METHOD

Dogs were used in all experiments. They were profoundly anesthetized by sodium barbital (0.3 gram per kilogram of body weight administered intravenously) and gave no evidence of pain during the experiments, at the completion of which they were killed. After making a long midline abdominal incision, the intestines were traumatized (2) by passing them continuously between the fingers. The trauma resulted in a copious weeping of fluid from the peritoneal surfaces. The intestines were not withdrawn from the peritoneal cavity during the traumatization and the fluid that accumulated there was collected frequently for study.

The blood pressure was determined by placing in the carotid artery a cannula that was connected to a mercury manometer. Blood for the various analyses was withdrawn from one of the femoral veins and it was replaced by an equal quantity of blood that was obtained from a normal dog. Samples of blood were obtained before the trauma was begun and at approximately one and one-half hour intervals thereafter until the blood pressure had declined to a low level with the exception of experiment T 17. Urine was collected, at the same time that the samples of blood were withdrawn, through a catheter that was placed in the bladder. The animal was kept warm by the use of a heating pan.

The nitrogen content of the blood serum, the peritoneal fluid and the urine was determined. In the studies on the blood serum and the peritoneal fluid, the nitrogen was partitioned in most of the experiments. The nonprotein nitrogen was estimated by the method of Folin and Wu (3) except in those experiments in which the effects of concentrated solutions of glucose or gum acacia were being studied, in these the macro-Kjeldahl method was used. Albumin and globulin were separated by the use of 22.2 per cent sodium sulphate as recommended by Howe (4). Two cc samples of serum and fluid were analyzed instead of 0.5 cc samples and the dilutions with the sodium sulphate solutions were proportionately the same. Fifty of the resulting 62 cc were chosen as the aliquot parts. The nitrogen of the albumin and of the total protein were determined by the Gunning (5) modification of the Kjeldahl method. The total nitrogen of the urine was also determined by this method. In all the tables the nitrogen is expressed as protein.

Van Allen tubes were used in the hematocrit determinations. The method of Cohen and Smith (6) was employed for the estimation of the hemoglobin. The initial blood volume of the animal was assumed to be 10 per cent of the body weight and on this basis the original volumes of red blood cells and plasma were calculated by the use of the hematocrit readings. In some experiments both hemoglobin and hematocrit determinations were performed, while in others the hemoglobin was not determined. If the hemoglobin and red blood cell count were both determined, the alterations in the total blood volume were assumed to vary in an inverse ratio to the changes in the percentage of hemoglobin. Having determined the alteration in the total blood volume, the volumes of red blood cells and plasma were calculated from the hematocrit readings. When only hematocrit determinations were performed, it was assumed that the volume of red blood cells remained constant throughout.

the experiments and the alterations in the volume of plasma were computed from the changes in hematocrit readings. The figures for the entire amount of protein were obtained by multiplying the percentage of protein per unit volume of serum by the total amount of plasma. No attempt was made to determine the alterations in the blood volume and in the entire amount of protein in the experiments in which the whole blood was injected. It is realized that the methods of calculation are only relatively accurate. The main error is introduced by the fact that the volume of red blood cells did not remain constant since some red blood cells escaped with the plasma into the peritoneal cavity and others accumulated in the traumatized area.

In the experiments in which fluid was introduced intravenously, it was injected at body temperature at a constant rate into either the external jugular or femoral vein. The amount of fluid that was given was approximately 10 cc per kilogram of body weight per hour. The following fluids were used in the different experiments: (1) 0.9 per cent salt solution, (2) 3.0 per cent salt solution, (3) 6.0 per cent glucose, (4) 20 per cent glucose, (5) 6.0 per cent gum acacia, (6) Evans' solution of 6.0 per cent gum acacia and 20 per cent glucose in 0.9 per cent salt solution, (7) blood serum and (8) whole blood.

In most of the experiments in which fluid was injected, the water content of the blood and of skeletal muscle was determined by drying the tissue at 105° C. to a constant weight. Blood was collected from the femoral vein. Skeletal muscle was obtained from the pectoral and one of the flexor muscles of the thigh at the beginning of the experiments and from symmetrical sites on the opposite side of the body at the completion.

## RESULTS

### I. CONTINUOUS TRAUMA TO THE INTESTINES. NO FLUID INJECTED

When the intestines were traumatized continuously the animals usually lived six or seven hours. The decline in blood pressure was more rapid towards the end of the experiment than at the beginning. The hematocrit determinations showed a progressive increase in the proportion of red blood cells to plasma throughout the experiments. The calculated volume of plasma decreased until at the completion of the experiments it was approximately one half of its original value. The calculated total protein content of the blood serum remained approximately the same throughout the experiments and the corresponding fluid from the peritoneal cavity on analysis showed a content in protein very nearly the same as that of the serum. The percentages of albumin and of globulin in the blood serum were altered very little during the course of the experiments. The content in albumin of the peritoneal fluid was greater than that of the blood serum, whereas the content in globulin of the peritoneal fluid was usually less than that of the serum. Since the volume of plasma was greatly diminished, the absolute amounts of total protein, albumin and globulin in the blood stream were considerably reduced.

The results of three typical experiments are given in Table I.

TABLE I  
*The effects of continuous intestinal trauma No fluid injected*

I specit number and weight	Time from beginning	Total protein			Albumin			Globulin			Blood volume			Hemato- crit	Mean blood pressure
		Serum	Fluid	For total serum volume	Serum	Fluid	For total serum volume	Red blood cells	Plasma	Whole					
T 6 19.6 kgm	Control	5.38		58.7	2.70		29.5	2.68		29.2	864	1092	1956	44.0	170
	1° 30'	5.09	5.05	42.6	2.41	3.13	20.2	2.68	1.92	22.4	864	837	1701	50.8	113
	3° 10'	5.29	5.25	37.8	2.80	3.14	20.0	2.49	2.11	17.8	864	713	1577	54.8	113
	5°	5.33	6.06	28.2	2.69	3.67	14.2	2.64	2.39	14.0	864	529	1393	62.0	88
	7° 15'	4.99	6.83	23.2	2.52	3.97	11.7	2.47	2.86	11.5	864	465	1329	65.0	40
T 2 13 kgm	Control	5.13		36.1	2.68		19.0	2.45		17.4	584	711	1295	15.1	160
	1° 15'	5.30	5.40	26.4	2.85	3.54	14.2	2.45	1.86	12.2	584	498	1082	58.8	86
	3° 25'	5.10	6.32	20.9	3.31	4.42	12.8	2.09	1.90	8.1	584	388	972	60.1	90
	5° 30'	5.95	6.21	21.5	3.05	4.66	11.0	2.90	1.55	10.5	584	361	945	61.8	48
	5° 50'	5.61			3.29			2.35							0
T 5 18.2 kgm	Control	4.67		52.6	2.37		26.7	2.30		25.9	690	1125	1815	38.0	135
	1° 45'	5.89	5.29	45.0	2.25	2.59	17.2	3.64	2.70	27.8	690	762	1452	47.5	100
	3° 15'	5.43	5.17	28.2	2.12	2.65	11.0	3.31	2.82	17.2	690	520	1210	57.0	103
	5° 15'	6.06	5.33	33.1	2.79	2.87	15.2	3.27	2.46	17.9	690	514	1234	55.9	104
	7° 5'	5.25	5.79		1.98	3.35		3.27	2.44						

## II CONTINUOUS TRAUMA TO THE INTESTINES CONTINUOUS INJECTION OF FLUID

The amounts of fluid injected into the veins of each of the animals in these experiments were excessive. However, determinations were performed frequently during the experiments and the effects of small as well as large amounts of fluid are to be seen in the results in the tables. The average survival period of the animals in these experiments was approximately the same as that in the experiments in which the intestines were traumatized and no fluid was introduced. The type of fluid that was injected in these experiments did not seem to alter considerably the length of life of the animals. The longest survival period was encountered in dog T 17 in which the intestines were traumatized and whole blood was injected for seven and one half hours. At the end of this time the blood pressure was essentially normal, but the animal died three hours later. The alterations in the percentage of hemoglobin paralleled fairly closely the changes in the hematocrit readings. In many experiments a dilution of the blood occurred just before death. In all experiments in which the nitrogen of the control specimen of urine was determined as well as that obtained later, it was found that the urine collected during the control period had the higher total protein equivalent. In most of the experiments the amount of nitrogen that was lost in the urine was very small. In all of these experiments the intestines were traumatized continuously.

The effects of the different solutions employed were briefly as follows. The detailed results of one experiment in each group are given in Table II. Because of lack of space, the others are not included.

*1 The injection of 0.9 per cent salt solution*

Four experiments of this type were performed. Associated with the trauma and the injection of large amounts of salt solution there was an increase in the proportion of red blood cells to plasma in all of the experiments. The calculated total volume of blood plasma decreased. The percentage of protein per unit volume of serum decreased markedly in all experiments except T 48, in which there was very little alteration. The calculated entire amount of protein in the blood stream at the completion of the experiments was approximately one-half of the original values. There was a decrease in the concentration of albumin and globulin. The content in albumin of the peritoneal fluid was greater than that of the blood serum while the globulin content of the serum was greater than that of the fluid.

*2 The injection of 3.0 per cent salt solution*

In three of the four experiments, the injection of 3.0 per cent salt solution while the intestines were being traumatized was associated with



TABLE II  
*The effects of continuous intestinal trauma and of the continuous intravenous injection of fluids*

Experiment number and weight	Time from beginning	Amount of fluid given	Total protein				Albumin				Globulin				Blood volume				Hematocrit	Mean blood pressure
			Serum	For total serum volume	Fluid	per cent	Serum	For total serum volume	Fluid	per cent	Serum	For total serum volume	Fluid	per cent	Red blood cells	Plasma	Whole			
cc	per cent	grams	per cent	per cent	grams	per cent	per cent	grams	per cent	cc	cc	cc								

Salt solution, 0.9 per cent																		
7 15	Control	0	5.96	63.5	5.21	3.06	32.6	3.17	2.90	30.9	860	1074	1931	44.5	145			
10 3	1° 15'	188	5.71	15.7	1.82	2.67	21.4	2.82	3.01	24.3	860	799	1659	51.8	106			
kgm	2° 15'	413	5.18	40.0	1.82	2.51	19.5	2.82	2.64	20.5	860	768	1628	52.8	95			
	1° 15'	638	4.83	28.9	1.24	2.39	14.3	2.52	2.44	14.6	860	598	1458	59.0	85			
	5° 30'	825	4.11	24.6	4.18	1.99	11.8	3.16	2.15	12.8	860	593	1453	59.2	53			
	6° 15'	1013	3.71	26.9		1.83	13.2		1.91	13.7	860	718	1578	51.5	30			

Salt solution, 3.0 per cent																		
7 21	Control	0	5.65	35.2	4.26	2.60	16.2	2.53	3.05	19.0	676	624	1300	52.0	156			
13	1° 30'	225	4.58	23.1	3.30	2.32	11.9	1.95	2.26	11.5	676	511	1187	57.0	110			
kgm	3° 30'	525	3.79	24.2	3.25	1.77	11.3	2.18	2.02	12.9	676	636	1312	51.5	92			
	5° 30'	825	3.68	18.8	3.25	1.46	7.5	2.18	2.22	11.3	676	511	1187	57.0	80			
	6° 50'	975	3.01	18.3	3.27	1.26	7.6	1.66	1.78	10.7	676	600	1276	53.0	11			

Glucose solution, 6.0 per cent																		
7 19	Control	0	5.75	44.4	4.95	2.89	22.4	2.60	2.86	22.2	526	774	1300	40.5	140			
13	1° 10'	250	4.50	38.6	3.80	2.22	19.1	2.17	2.28	19.6	526	858	1384	38.0	94			
kgm	3° 45'	563	3.91	36.5	3.75	1.86	17.3	2.17	2.08	19.3	526	927	1453	36.2	90			
	5° 30'	825	3.87	31.7	3.75	1.89	17.0	2.19	1.98	17.8	526	897	1423	38.3	71			
	8°	1200	3.27	31.9	4.21	1.63	15.9	2.77	1.64	16.0	526	977	1503	35.0	40			

TABLE II (continued)

Experiment number and weight	Time from beginning	Total protein			Albumin			Globulin			Blood volume			Mean blood pressure
		Serum	For total serum volume	Fluid	Serum	For total serum volume	Fluid	Serum	For total serum volume	Fluid	Red blood cells	Plasma	Whole	
		per cent	grams	per cent	per cent	grams	per cent	per cent	grams	per cent	cc.	cc.	cc.	mm. Hg
Glucose solution 20.0 per cent														
T 18	Control	0	6.26	48.6	3.59	27.9	3.41	2.67	20.7	1.41	623	777	1400	164
14	1° 40'	250	4.98	38.7	3.08	23.9	3.61	1.90	14.8	1.08	623	777	1400	136
kgm	3° 20'	500	4.38	30.2	2.50	17.2	3.61	1.88	13.0	0.97	623	689	1312	95
	5° 20'	800	3.63	28.8	2.12	16.8	2.85	1.51	12.0	0.91	623	792	1415	92
	7° 15'	1088	2.89	32.7	1.45	16.4	2.68	1.44	16.3	0.91	623	1130	1753	0
Glucose solution 20.0 per cent														
Gum acacia 6.0 per cent in 0.9 per cent salt solution														
T 28	Control	0	5.07	40.8	2.33	18.8	2.48	2.74	22.1	1.48	555	805	1360	124
13.6	1° 30'	225	4.15	34.8	1.81	15.2	2.10	2.34	19.6	1.45	555	839	1394	88
kgm.	3°	450	3.53	32.9	1.57	14.7	2.10	1.96	18.3	1.43	555	933	1488	103
	5°	750	2.76	29.0	1.20	12.6	1.57	1.56	16.4	1.35	555	1050	1605	65
	6° 20'	950	2.26	25.5	1.07	12.1	1.69	1.19	13.4	1.35	555	1126	1681	50
Gum acacia, 6.0 per cent, and glucose 20.0 per cent in 0.9 per cent salt solution														
T 9	Control	0	5.67	51.1	2.86	25.8	3.95	2.81	25.3	1.34	680	901	1581	144
15.8	1°	150	3.96	34.3	1.88	16.6	3.03	2.08	17.7	1.17	680	884	1564	125
kgm	2° 30'	375	3.20	31.0	1.52	14.7	2.37	1.68	16.3	1.08	680	970	1650	98
	3° 45'	563	2.85	25.6	1.55	13.9	2.15	1.30	11.7	1.08	680	901	1581	83
	5° 45'	863	2.12	21.6	0.71	7.2	2.15	1.41	14.4	1.08	680	1020	1700	50
	6° 45'	1013	1.98	31.1	1.08	17.0		0.90	14.1		680	1550	2230	0

TABLE II (continued)

TABLE II (continued)

Experiment number and weight	Time from beginning	Amount of fluid given	Total protein			Albumin			Globulin			Blood volume			Hematocrit	Mean blood pressure
			Serum	For total serum volume	Fluid	Serum	For total serum volume	Fluid	Serum	For total serum volume	Fluid	Red blood cells	Plasma	Whole		
			per cent	grams	per cent	per cent	grams	per cent	per cent	grams	per cent	cc	cc	cc	per cent	mm Hg
Blood serum																
T 27	Control	0	6.38	52.1	5.41	3.01	18.4	3.05	2.59	15.8	2.36	634	817	1452	43.7	160
115	1° 10'	250	5.60	34.2	5.54	3.24	19.4	3.25	2.73	16.3	2.29	634	610	1244	51.0	85
kgm	3° 35'	538	5.97	35.7	5.95	3.03	17.3	3.22	2.90	16.6	2.73	634	597	1231	51.5	85
	5° 35'	838	5.93	33.9					2.61				571	1205	52.6	15
	Injected serum		5.50													
Whole blood																
T 17	Control	0	4.41		6.00										47.3	150
66	1° 30'	99	5.28		5.11										55.5	65
kgm	3° 10'	209	6.00		5.37										64.0	58
	5° 15'	346	5.37		6.51										69.0	70
	6° 25'	412	6.00												67.5	0

Slight edema of lungs

T 21 Total urine 21 cc with a total protein equivalent of 2.33 grams  
 T 19 Total urine 104 cc with a total protein equivalent of 1.26 grams  
 T 20 Total urine 395 cc with a total protein equivalent of 6.2 grams  
 T 28 Total urine 77 cc  
 T 27 Total urine 23 cc with a total protein equivalent of 1.86 grams  
 T 17 Total urine 4 cc with a total protein equivalent of 0.33 gram

Slight edema of lungs

T 21 Total urine 21 cc with a total protein equivalent of 2.33 grams  
 T 19 Total urine 104 cc with a total protein equivalent of 1.26 grams  
 T 20 Total urine 395 cc with a total protein equivalent of 6.2 grams  
 T 28 Total urine 77 cc  
 T 27 Total urine 23 cc with a total protein equivalent of 1.86 grams  
 T 17 Total urine 4 cc with a total protein equivalent of 0.33 gram

an increase in the concentration of red blood cells. The calculated volume of plasma decreased in three of the four experiments. In all experiments there was a decrease in the percentage of total protein, of albumin and of globulin with a diminution in the calculated entire amount of each in the blood stream. The content in albumin of the peritoneal fluid was slightly greater than that of the blood serum, while the globulin content of the serum was greater than that of the fluid.

### *3 The injection of 60 per cent glucose solution*

In three of the four experiments there was an increase in the proportion of red blood cells to plasma. In one experiment the volume of plasma increased and in three it decreased. In all experiments there was a decrease in the percentage and in the calculated entire amount of total protein, albumin and globulin. The albumin content of the peritoneal fluid was usually greater than that of the blood serum, while the globulin content of the serum was greater.

### *4 The injection of 20 per cent glucose solution*

A concentration of the blood occurred during the early part of the three experiments. During the later stages of two of the experiments, a dilution of the blood took place. There was a marked diminution in the concentration of total protein, albumin and globulin in all instances and the calculated entire amount of each in the blood stream was greatly decreased. There was a higher percentage of albumin and a lower percentage of globulin in the blood serum than in the peritoneal fluid.

### *5 The injection of 60 per cent gum acacia solution*

If six per cent gum acacia in normal salt solution was injected during the trauma, there was an increase in the concentration of the blood at the end of an hour and a half in two of the three experiments. Later observations showed a definite dilution of the blood. There was a marked decrease in the total protein, albumin and globulin per unit volume of serum. Even though there was an increase in the calculated total volume of plasma in most of the experiments the total amount of the protein constituents in the blood stream was markedly diminished. The amount of gum acacia that remained in the circulation was not determined.

### *6 The injection of 60 per cent gum acacia and 200 per cent glucose in 0.9 per cent normal salt solution*

In all experiments there was a marked dilution of the red blood cells and a large increase in the volume of plasma in the blood stream. The percentages of total protein, of albumin, and of globulin per unit volume of serum and the calculated entire amounts of each in the blood stream were decreased in all experiments. The concentration of albumin in the

peritoneal fluid was greater and the concentration of globulin was less than in the blood serum

### 7 *The injection of blood serum*

Even though blood serum was injected continuously, the concentration of red blood cells increased in two of the three experiments. The content of total protein, of albumin, and of globulin in the blood serum remained approximately the same throughout the course of the experiments. In two of the three experiments there was a decrease in the calculated entire amount of protein in the blood stream. Since protein was present in the blood serum that was being injected, the true loss of protein was greater than the apparent loss. For example, in experiment T 27, the calculated entire amount of protein in the blood stream before the trauma was instituted was 52 grams. Five and one-half hours later it was 34 grams, indicating a loss of 18 grams. But during this time 46 grams of protein had been injected so that the true loss was 64 grams rather than 18.

### 8 *The injection of whole blood*

The concentration of the red blood cells increased tremendously in the three experiments in which whole blood was injected. No attempt was made to estimate the alterations in the volume of circulating blood but we can be quite certain that the volume of plasma was reduced even though whole blood was injected. The concentration of total protein, albumin and globulin in the blood serum either remained the same or increased slightly during the course of the studies. The results of these experiments differ from those obtained after the injection of blood serum. There was a much more decided increase in the concentration of the red blood cells.

### WATER CONTENT OF BLOOD AND MUSCLE

As has been stated previously, the water contents of blood and of skeletal muscles were determined in experiments in which the intestines were traumatized and various fluids were injected. The effects of intestinal trauma alone had been determined in previous experiments by Harris and Blalock (7). In the experiments in which normal salt solution was introduced there was a slight decrease in the water content of the blood. In those in which gum acacia solutions were used there was a definite increase in the water content of the blood and in those in which whole blood was injected there was a rather large decrease. The alterations that were found in the experiments in which 3.0 per cent salt solution, 6.0 per cent glucose, 20.0 per cent glucose and blood serum were injected were negligible. In the experiments in which the solution of gum acacia and glucose was injected a definite increase in the water content of muscle was found. When 20.0 per cent glucose was injected, a slight

decrease resulted. With these two exceptions the alterations in the water content of skeletal muscle were of too small magnitude to be of importance.

The results of the determinations on the water content of blood and muscle in the various experiments are given in Table III.

### DISCUSSION

When the intestines are passed continuously between the fingers, a copious weeping of fluid from the peritoneal surfaces results. In a previous communication (8) studies of the composition of this fluid were reported. It was found among other things that the protein content of the fluid was approximately the same as that of the blood plasma. Those studies did not include determinations of the albumin and globulin content of the fluid. It has been found in this study that the albumin content of the peritoneal fluid was greater than that of the blood serum while the globulin content of the fluid was less than that of blood serum. When fluids were injected continuously while the intestines were being traumatized, usually this same relationship between the blood serum and the peritoneal fluid was found. It is doubtful if the high percentage of albumin in the fluid was due entirely to concentration as a result of evaporation. Possibly trauma resulted in the loss of some of the proteins of the fixed tissues into the fluid. If it is assumed that the osmotic pressure in the capillaries and in the tissue spaces of the injured intestines were the same, then it is apparent that there would be no force resisting the filtration pressure in the vessels of the damaged area and that large amounts of fluid would be expressed.

It is of particular interest in these experiments that the percentage of protein, albumin and globulin per unit volume of serum remained practically constant throughout the period in which the intestines were traumatized and no fluid was injected and that it decreased considerably in most of the experiments in which fluids other than blood and serum were introduced. A careful review of the literature did not reveal any experiments closely similar to the present studies. White and Erlanger (9) determined in three experiments the effects of the injection of a strongly hypertonic solution of gum acacia and glucose into animals in shock as a result of partial occlusion of the inferior vena cava. In their experiments the injection was not continuous and the effects of only one solution were determined. In summarizing their results, they state, "The blood volume, markedly diminished in shock, is increased to above its normal level by the injection and then falls to or below its normal level. The absolute amount of plasma protein is markedly diminished in shock, is increased by the injection and the increase continues for some time after the injection. It is believed that at least a part of the increase in plasma protein following the injection in shock is due to a passage of protein in through the vessel walls."

TABLE III

*The effects of continuous intestinal trauma and the continuous administration of fluids on the water content of blood and muscles*

Fluid given	Time	Amount of fluid given	Blood pressure	Hemato-crit	Water		
					Blood	Pectoral muscle	Thigh muscle
<i>per cent</i>		<i>cc.</i>	<i>mm Hg</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
0.9 Saline	Control 6° 45'	0 1013	145 30	44.5 54.5	80.40 77.50	74.05 75.40	73.15 72.60
0.9 Saline	Control 3° 15'	0 488	156 48	51.2 53.0	79.20 77.60	75.40 75.20	74.35 74.00
0.9 Saline	Control 7° 30'	0 1260	130 78	50.5 62.3	77.80 76.20	76.30 75.80	74.55 74.20
0.9 Saline	Control 7°	0 1386	160 22	52.4 60.7	76.40 75.30	70.60 75.25	68.95 67.40
Average	Control Later		148 45	49.65 57.63	78.45 76.65	74.09 75.41	72.75 72.05
3.0 Saline	Control 6° 50'	0 975	156 44	52.0 53.0	77.20 79.42	73.35 75.60	73.00 72.00
3.0 Saline	Control 5°	0 750	136 30	33.0 30.4	83.00 85.30	77.18 78.70	75.05 78.25
3.0 Saline	Control 7°	0 1148	128 0	49.3 54.2	78.90 79.10	77.55 74.30	74.95 74.60
3.0 Saline	Control 7° 30'	0 1095	160 105	53.9 61.3	76.20 75.90		70.70 69.50
Average	Control Later		145 45	47.0 49.7	78.83 79.93	76.03 76.30	73.43 73.59
6.0 Glucose	Control 8°	0 1200	140 40	40.5 35.0	80.50 84.30		63.15 67.20
6.0 Glucose	Control 4°	0 600	100 35	33.2 37.5	83.30 83.05	78.30 76.58	75.58 76.62
6.0 Glucose	Control 6° 40'	0 707	130 21	41.7 38.0	80.95 83.00	75.60 76.10	73.55 75.00
6.0 Glucose	Control 7°	0 896	152 100	45.4 54.0	79.50 78.10	76.40 75.00	74.80 73.70
Average	Control Later		131 49	40.2 41.1	81.06 82.11	76.77 75.89	71.77 73.13
20.0 Glucose	Control 7° 30'	0 1125	130 40	43.2 59.0	78.90 75.45	73.90 73.20	72.65 72.60
20.0 Glucose	Control 7° 15'	0 1088	164 0	44.5 35.5	79.00 81.75	75.62 74.30	73.88 72.10
20.0 Glucose	Control 7° 30'	0 848	130 62	48.8 36.8	78.25 80.30	75.55 71.20	74.60 69.65

TABLE III (continued)

Fluid given	Time	Amount of fluid given	Blood pressure	Hematocrit	Water		
					Blood	Pectoral muscle	Thigh muscle
<i>per cent</i> Average	Control Later	<i>cc.</i>	<i>mm Hg</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
			141 34	45.5 43.8	78.72 79.17	75.02 72.90	73.71 71.45
60 Acacia	Control 6° 20'	0 950	124 50	40.8 33.0	80.90 83.30	78.25 78.30	77.25 77.62
60 Acacia	Control 5° 15'	0 788	150 0	39.8 36.0	80.10 82.70	75.55 76.60	70.30 64.90
60 Acacia	Control 7° 30'	0 975	140 90	38.8 31.7	80.00 83.80	75.80 77.75	74.85 75.00
Average	Control Later		138 47	39.8 33.6	80.33 83.27	76.53 77.55	74.13 72.51
Acacia, glucose saline	Control 6° 15'	0 938	154 0	53.0 25.5	77.70 84.00	75.45 71.78	74.85 71.70
Acacia, glucose saline	Control 6° 45'	0 1013	144 0	43.0 30.2	79.50 82.23	70.15 66.15	72.00 68.80
Acacia, glucose, saline	Control 7° 30'	0 840	150 38	42.8 30.0	80.75 83.75	77.60 75.20	73.58 69.90
Acacia glucose saline	Control 7°	0 742	130 63	43.0 33.3	79.95 82.45	75.20 75.50	74.25 70.60
Average	Control Later		145 25	45.5 29.8	79.48 83.36	74.60 72.16	73.67 70.25
Blood plasma	Control 5° 35'	0 838	160 15	43.7 52.6	79.10 77.50	77.40 76.30	74.40 73.35
Blood plasma	Control 5 25'	0 444	123 73	42.2 40.0	80.50 81.05	75.20 75.00	72.40 72.40
Blood plasma	Control 4° 45'	0 427	133 0	45.1 47.0	79.70 79.40	76.5 76.2	74.20 72.50
Average	Control Later		139 29	43.7 46.5	79.77 79.32	76.37 75.83	73.67 72.75
Whole blood	Control 4° 5'	0 613	146 0	39.3 59.0	82.20 77.10	70.20 74.90	69.15 67.30
Whole blood	Control 7° 35'	0 1138	168 136	44.0 76.5	79.30 71.10	76.10 75.60	74.85 73.10
Whole blood	Control 6 25'	0 412	150 0	47.3 67.5	79.35 74.50	77.15 75.40	74.80 73.15
Average	Control Later		155 45	42.9 67.7	80.28 74.23	74.48 75.30	72.93 71.18



It is rather surprising in the experiments in which solutions of salt or of glucose were injected and in which there was a decrease in the amount of total protein, albumin and globulin per unit volume of serum that there was usually at the same time a decrease in the volume of plasma in the blood stream. The smallest alterations in the plasma volume were associated with the giving of 20 per cent glucose solution. Since the plasma volume was usually reduced, the decrease in the percentage of protein was not due to simple dilution by the injected fluid. As both the percentage of protein and the volume of plasma were usually both reduced, the entire amount of protein in the blood stream was greatly diminished. In the experiments in which the six per cent gum acacia or the gum acacia-glucose solutions were injected, the findings were different in that they were usually associated with an increase in the plasma volume and the decrease in the percentage of protein was greater than that usually found if salt or glucose solutions were used. The gum acacia-glucose solution produced a greater increase in the volume of plasma than did the six per cent gum acacia. Even though the plasma volume was greatly increased in some of these experiments, there was always a decrease in the entire amount of protein. The nitrogen content of the gum acacia that was used was found to be negligible. The values for protein in these experiments are misleading in that they do not give a true estimate of the osmotic pressure that the serum was capable of exerting since six per cent gum acacia has an osmotic pressure approximately the same as that of blood serum. The gum acacia that was present in the blood stream at any given time was not determined.

The results obtained in the studies of the effects of the injection of blood serum and whole blood differed from the others mainly in that the concentration of protein in the serum of the blood stream did not decrease. In two of the three experiments in which blood serum was introduced there was a decrease in the plasma volume and in the entire amount of serum protein, although enormous amounts of serum had been injected. In the experiments in which whole blood was administered, there was a tremendous increase in the concentration of the red blood cells and probably a decrease in the plasma volume.

It has been known for a long time that solutions of crystalloids leave the blood stream shortly after having been injected. That the injection of these solutions in the presence of gross capillary injury following trauma would result in a great decrease in the protein content of the blood plasma as well as in the volume of plasma has not been appreciated. Since the osmotic pressure in the vessels is maintained largely by the protein, the significance of the alterations is apparent. If the blood pressure and the plasma volume were the same in two animals one of which had and one of which had not received injections of solutions of crystalloids in large quantities, it is believed that the chances of recovery would be greater in

the animal that had not received the fluid since the protein content of the serum would not be decreased. This point could not be determined in these experiments as all of the animals died as a result of the long duration of the trauma and the depth of the anesthesia. Very little if any difference in the rapidity of the fall of the blood pressure was observed in the experiments on intestinal trauma alone and those in which both trauma and the injection of fluid were carried out. Perhaps the survival period of the latter group was a little longer but the difference was not great. In an animal in which the intestines had been traumatized for a long period, if the injection of crystalloid solutions was stopped, we have the distinct impression that the blood pressure declined more rapidly than in an animal in a similar condition in which no fluid had been introduced. We do not mean to imply if a patient is in shock as the result of an injury and no donor is obtainable that saline or similar solutions should not be injected. However, in the absence of a favorable response in the blood pressure after a considerable amount of solution had been injected, almost certainly the further administration of the same fluid intravenously would diminish the chances of recovery.

As has been stated previously, the total quantity of fluid injected in most of the experiments was quite large but frequent analyses were performed and in this way the effects of small amounts were also determined. There seems to be a tendency at the present time to use very large quantities of fluids intravenously in treating patients. In reporting their results with the use of normal salt solution in treating shock, MacFee and Baldrige (10) recently stated, "The solution has been given intravenously in amounts ranging from 2000 cc. to 8000 cc. at a single injection."

The gum acacia solutions that were injected presented the distinct advantage over the solutions of crystalloids in that they were associated with an increase in plasma volume rather than a decrease. It is quite true that the protein content of the serum was decreased to a lower value in these experiments than in any others, but the functions of the protein in maintaining the osmotic pressure of the circulating blood were probably taken over at least partially by the gum acacia which is a colloid and hence exerts an osmotic pressure. We do not know how much gum acacia was in the circulation at any one time or how long it remained there. Concerning the fate of gum acacia, Bayliss (11) states, "I have obtained the pentose test in the blood twenty-four hours after injection, but it had disappeared in three weeks. If eliminated in the urine, the process is very slow, since the urine collected for six hours after injection gave no reaction, even when concentrated."

When whole blood was injected continuously while the intestines were being traumatized, there was a tremendous increase in the concentration of the red blood cells. This was to be expected since the loss of

red blood cells at the site of the trauma was small as compared with the loss of plasma. The effects that may result when the blood becomes greatly concentrated have been described by Underhill in numerous publications.

The injection of blood serum was associated with the smallest alterations in the composition of the blood. The percentage of protein per unit volume of serum and the plasma volume were not changed greatly in these experiments. There was a large loss of protein in the fluid that escaped but due to the fact that serum was being injected, the lost protein of the blood serum was replaced.

The greatest part of the fluid that was lost from the circulation escaped through the capillaries of the injured area. Determinations of the water content of skeletal muscle showed that it did not escape into them. In most instances in which 3.0 per cent salt solution was injected, the dogs passed fluid from the rectum. The amount of fluid and of nitrogen that escaped in the urine was not very great in most of the experiments. Edema of the lungs was noted several times at autopsy.

### SUMMARY

All of the experiments were performed on dogs that were deeply anesthetized by sodium barbital. Shock was produced by continuously passing the intestines between the fingers. Blood serum and the fluid that escaped into the peritoneal cavity were collected frequently for the studies.

In the first group of experiments studies were performed on the blood serum and the peritoneal fluid during continuous trauma to the intestines. No fluid was administered. Among the findings were the following: (1) There was a great diminution in the volume of plasma in the circulation. (2) The protein content per unit volume of serum remained approximately the same throughout the experiments. (3) The total protein content of the blood serum and the peritoneal fluid were approximately the same in all instances. (4) The content in albumin of the peritoneal fluid was usually greater than that of the blood serum and the content in globulin was less.

In the second group of experiments various solutions were injected intravenously at a constant speed while the intestines were being traumatized. The solutions included (1) 0.9 per cent saline, (2) 3.0 per cent saline, (3) 6.0 per cent glucose, (4) 20.0 per cent glucose, (5) 6.0 per cent gum acacia, (6) a solution of 6.0 per cent gum acacia and 20.0 per cent glucose in normal saline, (7) blood serum and (8) whole blood. Among the findings were the following: (1) The total protein content of the blood serum and the peritoneal fluid that were collected simultaneously was approximately the same in all experiments. (2) The albumin content of the peritoneal fluid was usually greater than that of the blood.

serum while the globulin content was less (3) The injection of the solutions of salt and glucose was usually associated with a decrease in the percentage of protein per unit volume of serum, a diminution of the volume of plasma in the circulation and a great decrease in the absolute plasma protein (4) When the solutions of gum acacia were injected, there was a decrease in the percentage of protein, an increase in the plasma volume and a decrease in the absolute amount of plasma protein (5) The injection of blood serum was associated usually with comparatively small alterations in the percentage of protein, the volume of plasma and the absolute amount of plasma protein (6) When whole blood was injected, there was very little alteration in the percentage of protein in the blood serum Since there was a tremendous increase in the concentration of the blood, there was probably a rather large reduction in the plasma volume and in the absolute amount of plasma protein

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## INTRAVENOUS INJECTIONS

### A STUDY OF THE EFFECTS ON THE COMPOSITION OF THE BLOOD OF THE INJECTION OF VARIOUS FLUIDS INTO DOGS WITH NORMAL AND WITH LOW BLOOD PRESSURES

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In a previous study (1) in which the composition of the blood was determined in experiments in which fluid was injected continuously while the intestines were being traumatized, it was found that there was a great decrease in the protein content as well as in the volume of the blood plasma. The object of the experiments reported in this paper is to determine the effects of the intravenous injection of fluids on the composition of the blood of normal dogs

After a few experiments had been performed, it was observed that the results following the injection of the same type of solution were somewhat variable. For example, in some experiments the concentration of the red blood cells increased while in others it decreased. In the experiments in which the arterial pressure remained normal the results obtained were different from those if the pressure declined, even though the drop was only temporary.

We do not know why there was a decline in the blood pressure in many of the experiments. No trauma was instituted in any of them. It seems most likely that it was usually caused by the fluid that was being injected but this was not always the case as in some experiments the decline appeared before the introduction was begun. Probably the morphine or the barbital that was used as anesthetic caused the decline in some experiments. The dosage of these was not great, being only sufficiently large to maintain the animals quiet and free from pain. In many of the experiments the decline in pressure appeared almost simultaneously with the beginning of the introduction of fluid. It frequently returned later to its previous normal level. Vincent and Thompson (2) have described the effects on the arterial pressure of the injection of salt solution into the veins of decerebrate cats. They found that the injection of 10 cc. of salt solution in some instances produced an elevation or a decline in the pressure equivalent to as much as 60 mm Hg. The temperature of the fluid was thought to be the most important factor in determining the response.

## METHODS

Dogs were used in all experiments. Morphine or sodium barbital was used as the anesthetic. The results did not seem to be influenced by the anesthetic. After the animals became quiet a cannula that was connected to a mercury manometer was placed in the carotid artery in order to determine the blood pressure. Samples of blood were obtained from the femoral veins. The blood volume was determined at the beginning of most of the experiments by the dye method as outlined by Rowntree, Brown and Roth (3). The blood that was withdrawn was replaced in all instances by an equal quantity of blood obtained from another dog. In most experiments tests were performed in order to be sure that the blood of the donor and recipient were not incompatible. Urine was collected through a catheter in the bladder. Small pieces of muscle were obtained at the beginning and end of some of the experiments for determinations of the water content.

When all of the specimens for the control determinations had been obtained, fluid was introduced at a constant speed and at body temperature through a cannula that had been placed in either the external jugular or the femoral vein. The fluid was usually injected at the rate of 10 cc. per kilogram of body weight per hour. In most of the experiments the duration of the introduction of the fluid was four hours, and the various samples for the analyses were collected one hour, two and one-half hours and four hours after the beginning of the injection. At one and three hour intervals after the termination of the injection, more samples were obtained. In a few of the experiments fluid was introduced throughout the entire period of observation and specimens for the analyses were collected at approximately two hour intervals. The following fluids were used in the different experiments, (1) 0.9 per cent salt solution, (2) 3.0 per cent salt solution, (3) 6.0 per cent glucose, (4) 20.0 per cent glucose, (5) 6.0 per cent gum acacia, (6) Evans' solution of 6.0 per cent gum acacia and 20 per cent glucose in normal saline, (7) blood serum and (8) whole blood.

The hematocrit determinations were performed by the use of the Van Allen tubes. The method of Cohen and Smith (4) was employed for the estimation of the hemoglobin. The initial blood volume was determined in most of the experiments by the dye method and the values obtained are placed in brackets in the tables. When not determined the blood volume was assumed to be ten per cent of the body weight and the volumes of red blood cells and plasma were calculated from the hematocrit readings. The alterations in the whole blood, red blood cell and plasma volumes that occurred in the experiments were estimated in the following manner. When both hemoglobin and hematocrit readings were obtained, the alterations in the total blood volume were assumed to vary in an inverse ratio to the changes in the percentage of hemoglobin, and from the total blood volume the quantity of red blood cells was calculated from the hematocrit readings. In the few experiments in which the hemoglobin was not determined, it was assumed that the volume of red blood cells remained constant throughout the experiments and alterations in the plasma volume were computed from the changes in the hematocrit readings. The various determinations were performed on the blood that was injected to replace that removed for the studies. Any differences between that removed and that injected were not considered in the various calculations. This introduced very little error. In the experiments in which the effects of the injection of whole blood were studied, the calculations were slightly more complicated. If blood is used, one is adding a fluid that contains red blood cells and consequently the changes in the hematocrit readings and hemoglobin are not measures of al-

terations in the blood volume. The calculations were made in the following manner. From the initial whole blood volume, the succeeding volume was calculated by the use of the percentage of hemoglobin as in other experiments. The actual volume of blood injected was reduced to that volume which would represent a blood with an hematocrit reading the same as the circulating blood at the time of the injection. The resulting value was then added to the tentative volume calculated initially from the hemoglobin changes and the sum was considered as the true blood volume. Each succeeding volume was calculated not from the control volume but from the one immediately preceding it since the hematocrit reading was constantly changing.

The total protein, the albumin and the globulin were determined by the same methods used in the experiments described in a previous paper (1). The absolute amounts of the protein constituents were obtained by multiplying the percentage of each by the plasma volume. The water content of blood and of muscle was determined by drying them to a constant weight.

## RESULTS

The results of the experiments with the different solutions that were used will be described separately. These groups will be subdivided into those in which the blood pressure remained at approximately the normal level throughout the experiments and those in which there was a definite decline in the pressure. Because of lack of space, only one experiment of each type will be given in detail in the tables.

### I THE EFFECTS OF THE INJECTION OF NORMAL SALT SOLUTION

#### *A Essentially normal blood pressure*

Two experiments of this type were performed. During the injection of the fluid there was a decrease in the concentration of the red blood cells and a diminution of protein per unit volume of blood serum. After the injection was terminated, there was a return towards the previous control level. The volume of plasma increased slightly during the injection and decreased later. In one of the experiments at no time was there any appreciable alteration in the absolute amount of protein in the circulation. In the other a decrease in the entire amount of protein was found during the last half of the experiment. The alterations in the albumin and globulin content of the serum paralleled closely those in the total protein. The results of one of these experiments are given in Table I. In this and in the succeeding tables "Injected blood" refers to that which was introduced in order to replace that withdrawn for the analyses.

#### *B Low blood pressure*

Two experiments of this type were performed. The blood pressure in each declined early and never returned to the original control level. Fluid was injected during the entire duration of both experiments. There was an increase in the concentration of red blood cells, a decrease in the volume of plasma and a great decrease in both the percentage and abso-



TABLE I  
*The effects of the intravenous injection of normal salt solution*

The effects of the intravenous injection of normal salt solution

Experiment number and weight	Time from beginning	Amount of fluid given cc	Total protein		Albumin		Globulin		Blood volume			Hematocrit		Hemoglobin	Mean blood pressure
			Serum	1 or total serum volume grams	Serum	per cent	For total serum volume grams	Red blood cells cc	Plasma cc	Whole cc	per cent				
												per cent	grams		
Normal blood pressure															
I 64	Control	0	6.20	50.2	3.87	31.3	2.33	18.9	[665]*	[810]*	[1475]*	15.0	105.6	122	
151 kgm	1°	151	5.78	50.7	3.54	31.0	2.21	19.7	644	878	1522	42.3	102.3	116	
	2° 30'	375	5.63	50.2	3.31	29.5	2.32	20.7	643	891	1531	41.9	101.6	138	
	1°	601	5.29	19.6	3.15	29.5	2.14	20.1	655	935	1590	41.2	98.0	120	
	5°		5.68	52.6	3.32	30.8	2.36	21.8	636	924	1560	40.8	99.3	136	
	7°		5.75	50.6	3.34	29.7	2.41	20.9	648	888	1536	12.2	101.3	120	
	Injected blood		6.07		2.84		3.23					35.1	86.9		
Lowered blood pressure															
T 32 131 kgm	Control	0	5.30	39.0	2.37	6.3	1.82	4.8	602	736	1338	45.0		120	
	1° 30'	225	4.19	11.1	2.09	6.5	1.45	4.5	602	364	966	62.3		79	
	3° 30'	500	3.54	11.0	2.11	7.2	1.52	5.2	602	339	941	66.0		110	
	5° 30'	825	3.63	12.1	2.11	7.3	1.47	5.9	602	398	1000	64.0		76	
	7° 30'	1125	3.30	13.2	1.83				602			60.2		80	

\* Determined directly by the dye method

Protocol T 64 Morphine as anesthetic Total amount of urine collected during experiment was 180 cc with a total protein equivalent of 7.79 grams  
T 32 Morphine as anesthetic Total amount of urine collected during experiment was 162 cc with a total protein equivalent of 8.08 grams Died 6 hours later

lute amounts of total protein, albumin and globulin. It is interesting that almost the entire loss of protein occurred during the first part of the experiment at the time that the blood pressure declined so markedly. The results of one of these experiments are to be found in Table I.

## II THE EFFECTS OF THE INJECTION OF 3.0 PER CENT SALT SOLUTION

In no experiment in which 3.0 per cent salt solution was injected did the blood pressure remain entirely normal during the whole period of study. For this reason the experiments are divided into those in which the decline in blood pressure was great and those in which it was not so marked. In several instances bloody fluid was passed from the rectum.

### *A Fairly well sustained blood pressure*

Four experiments of this type were performed. There was a decrease in the concentration of red blood cells during the injection followed by a marked increase after the cessation of the injection. The plasma volume increased during the injection and declined markedly later. In the two instances in which the injections were continued throughout the experiments there was an increase in the hematocrit reading and a decrease in the plasma volume at the conclusion. The diminution in the percentage of total protein, albumin and globulin was usually progressive up to the time that the injection was stopped. During the early part of the experiments there was usually very little reduction in the absolute amount of plasma protein but the decline later was usually marked. The results of one of these experiments are enumerated in Table II.

### *B More marked decline in blood pressure*

Two experiments were performed. The findings are similar in most respects to those in the preceding group except that the changes were more pronounced. The loss of protein was greater in experiment T 38 in which a very marked decline in blood pressure occurred than in any other experiment reported in this paper. There was not only a large decrease in the concentration of total protein, albumin and globulin in the serum but also a great diminution in the plasma volume. The absolute amount of plasma protein was least when the blood pressure was lowest which was during the early part of the experiment. The results of one of these experiments are given in Table II.

## III THE EFFECTS OF THE INJECTION OF 6.0 PER CENT GLUCOSE SOLUTION

### *A Essentially normal blood pressure*

Two experiments of this type were performed. In one of these, T 65, there was no loss of protein. During the injection period, there was a slight increase in the plasma volume and a slight decrease in the percentage of protein. After the injection was terminated the plasma volume

TABLE II  
The effects of the intravenous injection of 3 per cent salt solution

Experiment number and weight	Time from beginning	Amount of fluid given	Total protein		Albumin		Globulin		Blood volume			Hematocrit	Hemoglobin	Mean blood pressure
			Serum	For total serum volume	Serum	For total serum volume	Serum	For total serum volume	Red blood cells	Plasma	Whole			
Fairly well sustained blood pressure														
T 60	Control	0	5.99	13.7	4.14	30.2	1.85	13.5	[813]*	[729]*	[1542]*	52.7	121.5	184
12.6	1°	126	5.28	38.9	3.38	21.9	1.90	14.0	830	736	1566	53.0	122.0	150
kgm	2° 30'	315	4.10	38.0	2.79	23.8	1.98	16.9	855	855	1710	50.0	112.4	105
	1°	501	4.10	39.2	2.55	24.4	1.95	18.6	874	956	1830	47.7	104.9	115
	5°		4.37	35.6	2.81	22.9	1.56	12.7	880	815	1695	51.9	113.2	130
	7°		1.20	30.6	2.62	19.1	1.68	12.3	921	729	1650	55.8	116.3	80
	Injected blood		5.25		3.38		1.87					36.2	78.5	
Lowered blood pressure														
T 18	Control	0	4.70	26.1	2.90	16.3	1.80	10.1	591	565	1153	51.3		130
11.5	1° 30'	225	3.16	7.5	1.94	4.6	1.22	2.9	591	235	826	71.5		65
kgm	3° 30'	525	3.16	10.1	1.84	5.9	1.32	4.2	591	318	909	65.0		100
	5° 30'	825	3.18	9.3	1.90	5.6	1.28	3.7	591	292	883	66.0		80
	7° 30'	1125	3.07	9.5	1.84	5.7	1.33	3.8	591	311	902	65.5		75

\* Determined directly by the dye method

Protocol, T 60 Barbitol is anesthetic. Total amount urine 400 cc with a total protein equivalent of 6.44 grams  
T 38 Morphine is anesthetic. Total amount of urine was 376 cc Passage of a large quantity of fluid from rectum

TABLE III  
The effects of the intravenous injection of 6 per cent glucose solution

Experiment number and weight	Time from beginning	Amount of fluid given	Total protein			Albumin			Globulin			Blood volume				Hematocrit	Hemoglobin	Mean blood pressure
			Serum	For total serum volume	Serum	For total serum volume	Serum	For total serum volume	Red blood cells	Plasma	Whole							
cc.	per cent	grams	per cent	grams	per cent	grams	cc.	cc.	cc.									

T 65 14 kgm.	Control	0	7.15	65.7	4.05	37.2	3.10	28.5	[710]*	[918]*	[1628]*	43.6	96.8	130
	1°	141	6.98	63.6	3.83	34.9	3.15	28.7	702	911	1613	43.5	97.7	130
	2° 30'	352	6.85	65.1	3.82	36.3	3.03	28.8	690	950	1640	42.1	96.1	130
	4°	564	6.58	66.2	3.62	36.3	2.96	29.9	698	1004	1702	41.0	92.6	130
	5°		6.99	68.9	3.80	37.4	3.19	31.5	694	986	1680	41.3	93.8	135
	7°		7.00	67.4	3.91	37.6	3.09	29.7	703	962	1665	42.2	94.6	125
	Injected blood		6.14		4.16		1.98					34.0	82.0	

T 59 13 kgm.	Control	0	6.14	58.5	3.28	31.2	2.86	27.2	[548]*	[952]*	[1500]*	36.5	82.0	150
	1°	130	5.28	55.1	2.94	30.7	2.34	24.4	556	1044	1600	34.8	76.9	90
	2° 30'	325	5.37	54.5	2.97	30.1	2.40	24.3	560	1015	1575	35.6	78.1	100
	4°	520	4.84	50.9	2.94	30.9	2.36	24.8	545	1052	1597	34.1	77.0	113
	5°		5.34	51.2	2.98	28.6	2.36	22.7	550	960	1510	36.4	81.5	122
	7°		5.20	50.3	3.06	29.6	2.14	20.7	549	967	1516	36.2	81.1	106
	Injected blood		5.64		3.33		2.31					35.8	80.9	

\* Determined directly by the dye method

Protocol. T 59 Sodium barbital as anesthetic. Total amount of urine 97 cc. with a total protein equivalent of 5.71 grams.

decreased and the percentage of protein increased. In the second experiment, T 78, there was a slight loss in the absolute amount of plasma protein even though the blood pressure remained at approximately the normal control level. This was encountered in very few of the experiments in which the blood pressure remained elevated. The results of one of these experiments are given in Table III.

### *B Decline in blood pressure*

Two experiments of this type were performed. In each there was a decline in the blood pressure during the early part of the experiment. Later the pressure approached but never reached that which was found in the control studies. In one experiment, T 59, there was a slight increase in the plasma volume, a decrease in the percentage of protein and a reduction in the absolute amount of plasma protein. In the other experiment, T 35, in which the mean blood pressure declined to 70 mm Hg simultaneously with the beginning of the injection and remained there for a short while, there was a great decrease in the plasma volume, percentage of protein and absolute amount of plasma protein. The results of one of these experiments are given in Table III.

## IV THE EFFECTS OF THE INJECTION OF 20.0 PER CENT GLUCOSE SOLUTION

### *A Normal blood pressure*

Two experiments of this type were performed. The findings in each are almost identical. The blood pressure remained at approximately the same level throughout the experiments. There was a slight decrease in the plasma volume and a slight increase in the percentage of total protein, albumin and globulin in the serum. In one experiment there was a slight increase in the absolute amounts of total protein, albumin and globulin in the circulation and in the other there was practically no alteration. The results of one of these experiments are to be seen in Table IV.

### *B Decline in blood pressure*

There was an early decline in the blood pressure in both of the experiments. During the injection there was a slight increase in the volume of plasma and a reduction in the percentage and absolute amount of plasma protein. After the introduction of fluid was terminated the percentage of protein in the serum increased but due to the fact that the plasma volume decreased, the absolute amount of plasma protein in the circulation did not rise appreciably. The results of one of these experiments are given in Table IV.

TABLE IV  
The effects of the intravenous injection of 20 per cent glucose solution

Experiment number and weight	Time from beginning	Amount of fluid given	Total protein		Albumin		Globulin		Blood volume				Hematocrit	Hemoglobin	Mean blood pressure
			Serum	For total serum volume	Serum	For total serum volume	Red blood cells	Plasma	Whole						
		cc.	per cent	grams	per cent	grams	per cent	grams	cc.	cc.	cc.	per cent	per cent	mm. Hg	
Normal blood pressure															
T 66	Control	0	6.44	58.4	2.39	21.6	4.05	36.8	[667]*	[908]*	[1575]*	42.3	96.6	126	
139	1°	139	5.25	55.7	2.03	21.6	3.22	34.1	657	1058	1715	38.3	88.8	132	
kgm.	2° 30'	348	5.52	58.7	2.15	22.9	3.37	35.8	646	1064	1710	37.8	89.0	132	
	4°	556	6.62	58.8	2.63	23.1	4.17	35.7	660	888	1548	42.6	98.3	130	
	5°		7.20	59.0	2.86	23.2	4.54	35.8	694	818	1512	45.9	106.0	132	
	7°		6.95	58.4	2.81	23.5	4.39	35.5	666	850	1516	44.0	100.3	128	
	Injected blood		5.70									26.9	66.4		
Lowered blood pressure															
T 61	Control	0	5.54	27.8	3.32	16.7	2.22	11.1	[311]*	[502]*	[813]*	38.3	93.2	130	
90	1°	90	3.49	20.2	2.06	11.9	1.43	8.3	308	578	886	34.8	85.5	50	
kgm.	2° 30'	225	3.36	21.9	1.84	12.0	1.42	9.3	311	652	963	32.3	75.8	63	
	4°	360	3.56	21.9	2.07	12.7	1.31	8.1	308	614	922	33.4	82.2	74	
	5°		4.33	23.3	2.46	13.2	1.71	9.2	326	538	864	37.7	87.8	54	
	7°		4.84	22.7	2.79	13.1	2.05	9.6	315	469	784	40.2	96.5	54	
	Injected blood				2.46							32.0	74.7		

\* Determined directly by the dye method

Protocols. T 66 Morphine as anesthetic. Total amount of urine 900 cc. with a total protein equivalent of 19.26 grams  
T 61 Barbitol as anesthetic. Total urine 66 cc with a total protein equivalent of 1.85 grams

TABLE V  
The effects of the intravenous injection of 6 per cent gum acacia solution

Experiment number and weight	Time from beginning	Amount of fluid given	Total protein			Albumin		Globulin		Blood volume			Hematocrit	Hemoglobin	Mean blood pressure
			Serum	For total serum volume	per cent	grams	Serum	For total serum volume	Red blood cells	Plasma	Whole				
		cc	per cent	grams	per cent	grams	per cent	grams	cc	cc	cc	per cent	per cent	mm Hg	
Essentially normal blood pressure															
T 77	Control	0	6.21	43.1	25.2	3.65	2.59	17.9	[388]*	[690]*	[1078]*	36.0	84.7	138	
138	1°	138	1.98	38.6	24.2	3.12	1.86	14.4	380	776	1156	32.9	79.0	110	
kgm.	2° 30'	315	4.18	37.2	22.7	2.51	1.64	11.5	383	892	1275	30.2	71.6	160	
	1°	552	3.77	40.2	25.2	2.37	1.40	15.0	390	1065	1455	26.8	62.7	168	
	5°		3.95	40.5	21.9	2.43	1.52	15.6	410	1025	1435	28.1	63.7	156	
	7°		3.95	15.6	27.7	2.40	1.55	17.9	335	1155	1490	22.6	61.2	128	
	Injected blood		5.17			2.48						27.1	64.7		
Lowered blood pressure															
T 71	Control	0	6.27	51.1	28.4	3.16	2.81	23.1	[665]*	[820]*	[1185]*	44.8	107.7	121	
11	1°	110	5.02	32.2	17.9	2.75	2.27	14.8	654	661	1315	49.7	120.5	100	
kgm	2° 30'	350	1.11	36.6	20.0	2.25	1.86	16.6	604	891	1495	44.5	107.1	121	
	1°	560	3.11	36.2					728	1062	1790	40.7	97.7	100	
	5°		3.61	31.8					663	957	1620	40.9	98.7	96	
	7°		3.68	30.8	17.0	2.03	1.65	13.8	658	837	1495	41.0	107.1	90	
	Injected blood		1.18			2.42	2.06					31.2	75.1		

\* Determined directly by the dye method

Protocols T 77. Morphine as anesthetic Shortly after first injection of fluid, blood pressure declined to a mean of 110 mm Hg but rose almost immediately Total urine 260 cc with a total protein equivalent of 7.16 grams  
T 71 Morphine as anesthetic Shortly before injection of fluid, mean blood pressure declined to 90 mm Hg and remained at that level for about 15 minutes

## V THE EFFECTS OF THE INJECTION OF 60 PER CENT GUM ACACIA SOLUTION

### *A Essentially normal blood pressure*

Several attempts were made before we were successful in performing an experiment in which the blood pressure did not decline markedly during the early part of the injection of the gum acacia solution. Except for boiling it, no effort was made to purify this solution. In the experiment that is reported the mean blood pressure declined to 110 mm. Hg simultaneously with the beginning of the injection but it rose almost immediately to its previous level. A marked increase in the plasma volume and a large decrease in the percentage of protein were associated with the giving of the gum acacia. The absolute amount of plasma protein decreased slightly. Following the cessation of the injection the volume of plasma did not decrease as was found in most of the experiments in which salt and glucose solutions were employed. At the end of the experiment the absolute amount of plasma protein was slightly greater than at the beginning. The results of this experiment are given in detail in Table V.

### *B Decline in blood pressure*

Only one complete experiment of this type was performed. At the end of the first hour of the injection there was a small but definite decline in the blood pressure and a decrease in the plasma volume. Following this the blood pressure rose and the plasma volume became greater than it had been during the control studies. There was a large drop in the protein content of the blood plasma and despite the fact that the volume of plasma increased, there was a great decrease in the absolute amount of plasma protein. The results of this experiment are to be seen in Table V.

## VI THE EFFECTS OF THE INJECTION OF GUM ACACIA GLUCOSE-SALINE SOLUTION

### *A Essentially normal blood pressure*

Two experiments of this type were performed. There was a great increase in the plasma volume and a marked decrease in the percentage of protein in the serum in both. During the early part of the experiments there was a small loss in the absolute amount of protein in the circulation in one and a small gain in the other. Later in one experiment an excessive amount of fluid was injected and the absolute amount of plasma protein diminished markedly. The results of one of these experiments are given in Table VI.

### *B Decline in blood pressure*

Two experiments of this type were performed. During the injection period in each there was an increase in the volume of plasma but the decrease in the percentage of protein was so great that there was a marked



TABLE VI

*The effects of the intravenous injection of gum acacia-glucose saline solution*

Experiment number and weight	Time from beginning	Amount of fluid given	Total protein			Albumin			Globulin			Blood volume				Hemoglobin per cent	Hematocrit per cent	Mean blood pressure mm Hg
			Serum	For total serum volume	Serum	For total serum volume	Serum	For total serum volume	Red blood cells	Plasma	Whole							
												per cent	grams	per cent	grams			
Essentially normal blood pressure																		
T 71	Control	0	7.77	19.6	5.12	32.7	2.65	16.9	[729]*	[638]*	[1367]*	53.3	120	130				
12.8 kgm	1°	128	5.63	11.8	3.18	27.7	2.13	16.9	705	795	1500	47.1	109.5	137				
	2° 30'	320	4.99	16.3	3.05	28.3	1.91	18.0	672	928	1600	41.2	102.1	110				
	1°	512	4.26	10.1	2.71	25.8	1.52	11.3	708	912	1650	42.9	99.3	114				
	5°		4.61	35.8	2.90	22.1	1.71	13.1	703	772	1475	17.7	111.1	115				
	7°		1.81	32.1	2.90	19.1	1.90	12.7	730	670	1100	52.2	117.1	60				
	Injected blood		5.96		3.17		2.19					12.7	102					
Lowered blood pressure																		
T 63	Control	0	5.10	37.9	2.78	20.6	2.32	17.3	[335]*	[713]*	[1078]*	31.2	75.2	157				
13.3 kgm	1°	133	3.17	21.8	1.65	12.9	1.52	11.9	316	781	1100	28.7	73.7	117				
	2° 30'	333	2.76	27.0	1.58	15.6	1.18	11.4	327	978	1305	25.1	62.1	116				
	1°	532	2.58	28.8	1.37	15.3	1.21	13.5	315	1115	1430	22.0	56.7	118				
	5°		2.78	26.9	1.57	16.1	1.81	10.8	327	968	1295	25.2	62.6	156				
	7°		3.06	28.1	1.65	15.1	1.11	13.0	319	918	1237	25.8	65.6	136				
	Injected blood		5.98		3.56		2.42					28.8	73.1					

\* Determined directly by the dye method

Protocols T 71

Morphine as anesthetic The mean blood pressure declined to 105 mm Hg immediately after first fluid was injected  
 It remained there for 5 minutes, then rose to 120 Total amount of urine was 322 cc with a total protein equivalent of 8.96 grams

T 63

Barbitol as anesthetic The mean blood pressure was 77 mm Hg when injection was begun Total amount of urine was 181 cc with a total protein equivalent of 6.54 grams

decrease in the absolute amount of plasma protein. After the introduction of fluid was terminated the plasma volume decreased, the protein content of the serum increased, and the absolute amount of plasma protein remained approximately the same as it had been at the time of the previous determination. The alterations in the amounts of albumin and globulin paralleled those in the total protein. In these experiments as in many of the others it is interesting that the greatest decrease in the amounts of total protein, albumin and globulin took place in the periods when the most marked decline in the blood pressure occurred. The results of one of these experiments are enumerated in Table VI.

## VII THE EFFECTS OF THE INJECTION OF BLOOD SERUM

### *A Normal blood pressure*

In the one experiment in which blood serum was injected in the presence of a normal blood pressure there was an increase in the plasma volume and in the content of total protein, albumin and globulin in the blood serum. Following the termination of the injection the plasma volume decreased slightly and the percentage of protein increased slightly. The absolute amount of plasma protein in the blood stream progressively increased during the injection and then decreased very slightly. If one subtracts from the absolute amount of plasma protein a figure that represents the amount of protein that was present in the serum that had been injected, it is to be seen that very little of the protein that was already in the vessels or that was introduced in the serum was lost from the blood stream.

The results of this experiment are given in Table VII. The figures for total protein, albumin and globulin that are placed in parentheses in the table indicate the entire amount that would have been present in the blood stream had protein not been present in the fluid that was injected. In other words, the figures in the parentheses were obtained by subtracting the amount that was injected from the amount that was actually present.

### *B Decline in blood pressure*

One experiment of this type was performed. The decline in the blood pressure appeared in the early part of the experiment and it was not very great. However, during the first hour of the experiment there was a tremendous decrease in the entire amount of protein in the blood stream despite the fact that protein was present in the fluid that was being injected. This decrease was due to the loss of plasma and not to a diminution in the percentage of protein. Later the plasma volume and the absolute amount of plasma protein increased, but if one makes allowance for the amount of protein that was injected, the loss was considerable. The results of this experiment are to be found in Table VII.

TABLE VII  
*The effects of the intravenous injection of blood serum*

Experiment number and weight	Time from beginning	Amount of fluid given	Total protein		Albumin		Globulin		Blood volume			Hematocrit	Hemoglobin	Mean blood pressure						
			Serum	For total serum volume	Serum	For total serum volume	Serum	For total serum volume	Red blood cells	Plasma	Whole									
												per cent	grams	per cent	grams	per cent	grams	cc	cc	cc
Normal blood pressure																				
1 70	Control	0	1 33	24 8 (23 3)†	3 16	18 2 (11 5)†	1 17	6 71 (9 8)†	[326]*	[574]*	[900]*	36 2	87 7	100						
86 kgm	1°	86	5 34	29 0 (25 2)†	3 30	17 6 (15 4)†	2 14	11 4 (7 3)†	324	544	868	37 4	90 9	108						
	2° 30'	214	5 64	39 4 (23 2)†	3 58	24 9 (13 0)†	2 09	14 5 (6 3)†	360	695	1035	31 1	83 1	104						
	1°	122	5 77	16 0 (22 5)†	3 54	28 2 (12 5)†	2 23	17 8 (6 1)†	341	795	1138	30 0	77 1	102						
	5°		5 81	15 3 (20 5)†	3 55	27 7 (12 2)†	2 26	17 6 (4 3)†	351	779	1130	31 1	77 7	99						
	7°		5 91	13 3	3 74	27 4	2 17	15 8	368	732	1100	33 4	79 6	98						
	Blood injected		6 66		3 79		2 87					54 8	132 7							
	Serum injected		6 66		3 79		2 87													

TABLE VII (continued)

Experiment number and weight	Time from beginning	Amount of fluid given	Total protein			Albumin			Globulin			Blood volume			Hematocrit	Hemoglobin	Mean blood pressure
			Serum	For total serum volume	Serum	For total serum volume	Serum	For total serum volume	Red blood cells	Plasma	Whole						
												per cent	grams	per cent			
Lowered blood pressure																	
T 72 14.5 kgm	Control	0	5.99	92.5 (61.3)†	3.47	53.6 (32.9)†	2.52	38.9 (25.5)†	[687]*	[1545]*	[2232]*	30.8	74.9	120			
	1°	145	6.19	68.2 (66.9)†	3.44	37.9 (32.6)†	2.75	30.3 (32.0)†	703	1102	1805	38.9	92.6	100			
	2 30'	363	6.68	86.9 (73.0)†	3.38	44.6 (40.2)†	3.30	43.6 (33.1)†	690	1320	2010	34.4	83.1	130			
	4°	580	6.75	105.3 (70.5)†	3.43	53.6 (39.1)†	3.32	51.8 (32.3)†	672	1560	2232	30.1	75.0	118			
	5°		6.83	102.5 (75.0)†	3.50	52.5 (41.6)†	3.33	50.0 (33.5)†	680	1500	2180	31.0	75.8	113			
	7°		7.07	107.0	3.65	55.0	3.44	52.2	705	1515	2220	31.6	75.4	120			
	Replaced blood Injected serum		5.64 5.53	2.79 2.31		2.85 3.22						25.2 70.1					

\* Determined directly by the dye method

† Indicates the entire amount that would have been present in the blood stream had protein not been present in the fluid that was injected

Protocols T 70 Morphine as anesthetic.

Total amount of urine 64 cc. with a total protein equivalent of 6.66 grams.

T 72 Morphine as anesthetic. Total amount of urine 185 cc. with a total protein equivalent of 7.31 grams.

Where two sets of figures are given, those in brackets were obtained by subtracting the amounts of protein that were injected from the quantity that was actually present in the blood stream

#### VIII THE EFFECTS OF THE INJECTION OF WHOLE BLOOD

##### *A Normal blood pressure*

Only one experiment was performed. There was an increase in the concentration of red blood cells, an increase in the percentage and in the absolute amounts of total protein, albumin and globulin in the serum. If one makes a deduction for the protein that was injected in the serum, there was a slight decrease in the absolute amount of plasma protein. These figures are placed in brackets in Table VIII in which the results appear.

##### *B Decline in blood pressure*

In the experiment of this type, the blood pressure declined to a mean of 95 mm Hg shortly before the beginning of the introduction of blood and it remained at that level for approximately one hour. There was a great increase in the concentration of red blood cells, a diminution in the volume of plasma, and a slight increase in the percentage of protein in the serum. Despite the fact that almost 17 grams of protein were present in all of the serum that was introduced the absolute amount of plasma protein was no greater at the end of the injection than during the control period. The results of this experiment are to be found in Table VIII.

In twenty of the experiments the water content of skeletal muscle was determined at the beginning and end of the studies. The results may be summarized briefly as follows. In the experiments in which isotonic solutions of salt or glucose were injected, there was a slight increase in the water content of muscle. In those instances in which hypertonic solutions of salt or glucose were used, there was usually a slight decrease in the water content of muscle. The injection of the gum acacia-glucose-saline solution was associated with a decrease in the water content of muscle. The results of these experiments are given in Table IX.

#### DISCUSSION

The results of these experiments may be divided into two rather definite groups. In those instances in which there was practically no alteration in the blood pressure, there was little or no loss in plasma protein. When solutions of salt or glucose were injected, they were lost from the blood stream rapidly and protein was usually not carried with them. When solutions containing gum acacia were injected, there was an increase in the plasma volume due to the fact that a large part of the solution remained in the blood stream. The percentage of protein was decreased but the absolute amount of plasma protein remained approximately the

TABLE VIII  
*The effects of the intravenous injection of whole blood*

Experiment number and weight	Time from beginning	Amount of fluid given	Total protein		Albumin		Globulin		Blood volume				Hematocrit	Hemoglobin	Mean blood pressure		
			Serum	For total serum volume	Serum	For total serum volume	Serum	For total serum volume	Red blood cells	Plasma	Whole						
												per cent				grams	per cent
Normal blood pressure																	
T 68	Control	0	5.92	38.6 (36.9)†	3.85	25.1 (25.0)†	2.07	13.5 (11.9)†	[463]*	[652]*	[1115]*	41.6	104.5	125			
10.3	1°	103	6.52	40.7 (34.6)†	4.25	26.5 (26.3)†	2.27	14.2 (8.3)†	505	624	1129	44.7	111.9	140			
kgm.	2° 30'	258	6.64	46.2 (29.9)†	4.32	30.0 (22.2)†	2.32	16.2 (7.7)†	568	695	1263	44.0	111.8	155			
	4°	412	6.66	45.2 (33.1)†	4.14	28.1 (23.7)†	2.52	17.1 (9.4)†	627	678	1305	48.1	119.0	166			
	5°		6.65	48.4 (29.1)†	4.06	29.6 (20.7)†	2.59	18.8 (8.4)†	621	728	1349	46.1	115.4	160			
	7°		6.66	44.4	3.99	26.6	2.67	17.8	633	667	1300	48.7	119.5	128			
	Injected blood		5.99		2.30		3.40					37.9	91.1				

TABLE VIII (continued)

Experiment number and weight	Time from beginning	Amount of fluid given	Total protein		Albumin		Globulin		Blood volume			Hematocrit	Hemoglobin	Mean blood pressure
			Serum	I or total serum volume	Serum	I or total serum volume	Serum	I or total serum volume	Red blood cells	Plasma	Whole			
												per cent	grams	per cent
Lowered blood pressure														
1 75	Control	0	5 16	32 1 (20 8)†	1 12	21 2 (16 0)†	1 31	7 9 (4 8)†	[444]*	[511]*	[955]*	46 5	111 1	161
11 5 kgm	1°	115	6 18	25 0 (16 6)†	1 56	18 1 (12 3)†	1 62	6 6 (1 3)†	473	401	877	53 9	132	110
	2° 30'	288	5 91	27 1 (11 5)†	4 09	18 7 (8 7)†	1 85	8 1 (2 8)†	540	457	997	51 2	130 1	112
	1°	160	6 28	28 3 (7 5)†	1 09	18 4 (6 3)†	2 19	9 9 (1 2)†	602	450	1052	57 3	136 3	130
	5°		6 11	24 3 (8 3)†	1 06	16 0 (7 3)†	2 08	8 3 (1 0)†	596	396	992	60 1	145	101
	7°		6 11	25 1	1 17	17 0	1 97	8 1	598	409	1007	59 1	112 8	117
	Injected blood		5 51		3 20							31	85 7	

\* Determined directly by the dye method

† Indicates the entire amount that would have been present in the blood stream had protein not been present in the fluid that was injected

Protocol 4 7 68

Morphine as anesthetic

Total urine 123 cc with 1 total protein equivalent of 11 23 grams

7 75

Morphine as anesthetic

Thirty minutes before the injection of fluid was begun, the blood pressure declined to a mean of 95 mm Hg and remained there for approximately one hour Total urine 16 cc with 1 total protein equivalent of 3 3 grams

TABLE IX

*The effects of the administration of fluids on the water content of blood and muscle—no trauma*

Fluid Injected	Experiment number	Time	Mean blood pressure	Hemato-crit	Water			Amount fluid given
					Blood	Pectoral muscle	Thigh muscle	
<i>per cent</i>			<i>mm. Hg</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>cc.</i>
0.9 Saline	T 31	Control 7° 30'	127 53	36.0 37.0	80.80 81.10	75.25 75.80	73.22 73.95	0 1125
0.9 Saline	T 32	Control 7° 30'	120 80	45.0 60.2	80.75 77.20	75.42 75.90	74.75 75.00	0 1125
0.9 Saline	T 41	Control 7° 5'	115 104	57.3 47.3	77.18 79.90	75.55 76.30	74.90 75.20	0 1091
0.9 Saline	T 58*	Control 7°	132 120	42.0 46.6	78.10 78.80	74.90 76.60	76.00 77.20	0 488
Average		Control Later	123.5 89.3	45.1 47.8	79.21 79.25	75.28 76.15	74.72 75.34	
3.0 Saline	T 33	Control 7° 5'	150 0	43.5 53.6	79.98 79.50	77.38 74.40	76.50 75.50	0 1063
3.0 Saline	T 42	Control 6° 45'	164 116	49.2 44.8	77.95 78.60	75.80 74.80	74.22 71.75	0 1094
3.0 Saline	T 38	Control 7° 30'	130 75	51.3 65.5	79.40 76.60	75.50 74.25	74.70 74.00	0 1125
3.0 Saline	T 60*	Control 7°	184 80	52.7 55.8	75.40 75.50	76.00 75.40	75.10 72.70	0 504
Average		Control Later	157 68	49.2 54.9	78.18 77.55	76.17 74.71	75.13 73.49	
6.0 Glucose	T 34	Control 7° 5'	146 150	37.0 40.0		76.20 81.30	73.95 75.80	0 1125
6.0 Glucose	T 35	Control 7° 30'	130 112	50.6 53.8	78.40 78.80	76.10 77.80	74.40 75.60	0 1125
6.0 Glucose	T 59*	Control 7°	150 106	36.5 36.2	80.20 81.40	77.80 78.10	75.50 76.90	0 520
Average		Control Later	142 123	41.4 43.3	79.30 80.10	76.70 79.07	74.62 76.10	



TABLE IX (continued)

Fluid injected	Experiment number	Time	Mean blood pressure	Hematocrit	Water			Amount fluid given
					Blood	Pectoral muscle	Thigh muscle	
<i>per cent</i> 20.0 Glucose	T 37	Control 7° 30'	mm Hg 120 122	<i>per cent</i> 45.4 46.2	<i>per cent</i> 79.60 79.80	<i>per cent</i> 79.50 75.00	<i>per cent</i> 75.60 73.50	cc 0 1125
20.0 Glucose	T 36	Control 7° 30'	160 108	38.0 40.6	81.40 81.40	77.00 75.50	75.60 75.30	0 1125
20.0 Glucose	T 61*	Control 7°	130 54	38.3 40.2	80.40 79.80	74.80 76.60	73.40 73.90	0 360
Average		Control Later	137 95	40.6 42.3	80.47 80.33	77.10 75.70	74.87 74.23	
6.0 Gum acacia	T 39	Control 7° 20'	152 111	46.3 35.0	78.6 82.6	75.70 75.60	79.70 75.40	0 1100
Acacia-glucose-saline	T 11	Control 6° 30'	164 60	52.0 35.0	76.77 81.90	68.05 64.95	65.40 62.75	0 975
Acacia-glucose-saline	T 13	Control 18° 50'	125 0	47.0 49.0	78.85 80.10	74.35 73.50	75.10 73.20	0 1250
Acacia-glucose-saline	T 12	Control 6°	128 20	58.0 49.0	75.05 78.60	67.20 54.40	67.30 69.75	0 900
Acacia glucose-saline	T 62*	Control 7°	190 96	44.5 43.3	78.10 78.00	77.20 74.80	74.90 73.40	0 508
Average		Control Later	152 44	50.4 44.1	77.19 79.65	71.70 66.91	70.68 69.78	
Whole blood	T 40	Control 7° 20'	154 165	43.0 51.0	79.80 76.90	76.80 76.30	75.20 75.20	0 1100

\* In experiments T 58, T 59, T 60, T 61, and T 62, the injection of fluid was discontinued after four hours and the samples were obtained three hours later. In the remaining experiments with the exception of T 13, fluid was injected during the entire duration of the experiments.

same. When blood serum or whole blood was injected, there was practically no loss either of the protein that was already in the blood stream or of that which was injected. In the other group in which there was a temporary or sustained decline in the pressure, the results were quite different. When solutions of salt or glucose were injected, there was a loss not only of a large part of the fluid that was injected but also a loss of blood plasma. It appeared in these experiments as though the mixture of the blood plasma and the injected solution passed through the vessel walls in the same composition as it existed in the blood stream. It seems

that the loss was similar in this respect if gum acacia, whole blood or blood serum were injected. The results were different in that gum acacia and blood are colloidal solutions and that the portion of the fluid which was left in the blood stream exerted an osmotic pressure. Although there was a decrease in both the percentage and absolute amounts of plasma protein following the introduction of gum acacia, the latter due to the size of its molecule probably compensated for part of the loss. No attempt was made to determine the amount of gum acacia in the serum. Due to the fact that the injected blood serum and whole blood had approximately the same content in protein as the plasma in the blood stream, their injection was associated with only the decrease in the absolute amount of protein and not with a reduction in the concentration of protein.

It has been known for a long time that solutions of crystalloids leave the blood stream shortly after having been introduced. Most of the evidence has indicated that the walls of the blood vessels are normally impermeable to the passage of proteins. White and Erlanger (5) found that the injection of a strongly hypertonic solution of glucose and gum acacia into normal dogs was associated with an increase in the plasma volume, a decrease in the percentage of plasma protein and very little alteration in the absolute amount of plasma protein. The blood pressure was not determined in these experiments. It is rather generally believed that plasma is lost from the blood stream in shock. In the preceding study it was found that the intravenous introduction of protein free fluids into dogs in which tissues had been injured is associated usually with a decrease both in the plasma volume and in the percentage of protein in the serum. The present experiments show that protein may be lost from the blood stream without gross injury to tissues. As to the mechanism of the loss, we can make no definite statements. Protein may have been lost because of the decline in the arterial pressure or both the decline in pressure and the loss of protein may have resulted from a common cause. It is possible that the passage of red blood cells from the spleen into the general circulation caused an increase in the concentration of red cells in the latter. That the spleen was not entirely responsible was demonstrated in two experiments in which the spleen was removed and normal salt solution was injected after a decline in blood pressure had been produced by the introduction of a small amount of gum acacia. In both instances a concentration of the blood and a marked loss of protein was found. The results of these experiments are given in Table X.

The observations on the water content of skeletal muscles showed that most of the fluid that left the blood stream did not pass into them. Analyses were not performed on other tissues but it seems likely that the greater part of the fluid escaped into the intraperitoneal structures. No free fluid was present in the peritoneal cavity in any of the experiments. In most instances in which 3.0 per cent salt solution was injected, there

TABLE V  
The effects of the intravenous injection of normal salt solution after removal of the spleen (Lowered blood pressure)

Experiment number and weight	Time from beginning	Amount of fluid given	Total protein		Albumin		Globulin		Blood volume			Hematocrit	Hemoglobin	Mean blood pressure
			Serum	For total serum volume	Serum	For total serum volume	Serum	For total serum volume	Red blood cells	Plasma	Whole			
		cc	per cent	grams	per cent	grams	per cent	grams	cc	cc	cc	per cent	per cent	mm Hg
T 101 135 kgm	Control	0	6.38	53.0	4.19	37.2	1.89	15.8	[550]*	[830]*	[1380]*	10.0	89.3	150
	1°	135	5.58	39.2	3.99	28.0	1.59	11.2	543	702	1215	43.6	99.0	116
	2° 30'	338	5.58	36.5	3.88	25.1	1.70	11.1	539	651	1193	45.2	103.4	118
	1°	510	5.58	35.3	3.95	25.0	1.63	10.3	512	633	1175	46.1	105.6	110
	5° 30'		6.28	35.0	1.53	25.2	1.75	10.8	536	558	1091	49.0	112.7	126
	7°		6.37	36.5	1.43	25.1	1.94	11.1	537	573	1110	48.1	110.0	105
	Injected blood		6.10		3.53		2.57					31.5	82.1	
T 103 111 kgm	Control	0	6.80	58.9	3.80	32.9	3.00	26.0	[426]	[866]	[1292]	32.9	73.1	168
	1°	111	5.98	51.2	3.65	31.2	2.33	20.0	415	855	1270	32.7	74.0	138
	2° 30'	360	5.60	18.8	3.18	27.7	2.42	21.1	120	872	1292	32.5	73.2	139
	1°	576	5.60	15.3	3.31	26.8	2.29	18.5	135	810	1215	31.9	76.2	130
	5° 30'		5.88	13.3	3.51	25.8	2.37	17.5	434	736	1170	37.2	81.1	123
	7°		6.00	43.8					433	730	1163	37.2	81.5	130
	Injected blood		5.57		2.74		1.83					39.1	89.4	

\* Determined directly by the dye method

Protocols Sodium barbital is anesthetic in both experiments

T 101 Shortly before the introduction of salt solution was begun the animal was given 6 cc of 6 per cent gum acacia solution in the external jugular vein. The blood pressure declined to a mean of 70 mm Hg, remained there for about 5 minutes and returned in 15 minutes to the previous normal level. Total urine 997 cc with a total protein equivalent of 18.1 grams.

T 103 After removal of the spleen, 10 cc of 6.0 per cent gum acacia were injected into the external jugular vein. The blood pressure declined to a mean of 86 mm Hg almost immediately. The injection of normal salt solution was then begun. Shortly thereafter the blood pressure rose rapidly. Total urine 537 cc with a total protein equivalent of 13.9 grams.

was a passage of fluid from the rectum. In several instances, the lungs were definitely edematous.

### SUMMARY

The effects on the composition of the blood of the injection of various solutions was determined in normal dogs and in dogs in which due to the anesthetic or unknown reason the blood pressure at some time during the experiments declined. No trauma was instituted in any of the experiments. The solutions that were injected in the different experiments included (1) 0.9 per cent salt solution, (2) 3.0 per cent salt solution, (3) 6.0 per cent glucose, (4) 20.0 per cent glucose, (5) 6.0 per cent gum acacia, (6) 6.0 per cent gum acacia and 20.0 per cent glucose in normal saline, (7) blood serum and (8) whole blood. The solutions were injected continuously usually at the rate of 10 cc. per kilogram of body weight per hour for four hours. Determinations were performed before, during and after the completion of the injections.

Some of the findings are summarized below.

I. Experiments in which the blood pressure remained at essentially the normal level.

(a) The injection of solutions of salt or glucose was usually associated with a slight increase in the volume of blood plasma, a slight decrease in the percentage of protein and very little alteration in the absolute amount of plasma protein in the circulation.

(b) When solutions containing gum acacia were injected, there was a definite increase in the plasma volume, a rather large decrease in the percentage of protein and very little alteration in the absolute amount of plasma protein.

(c) The introduction of blood serum or whole blood was associated with an increase in the plasma volume and in the percentage and absolute amounts of plasma protein.

II. Experiments in which there was a temporary or well sustained decline in the blood pressure.

(a) The injection of solutions of salt or glucose was usually associated with a decrease in the volume of plasma, a decrease in the percentage of protein and a marked diminution in the absolute amount of plasma protein.

(b) The introduction of solutions containing gum acacia was associated with very little alteration in the plasma volume, a great decrease in the percentage of protein and a reduction in the absolute amount of plasma protein.

(c) When whole blood or blood serum was injected the alterations in the plasma volume were not marked and there was a slight increase in the percentage of protein. If deductions are made for the protein that was present in the whole blood or blood serum that was injected, a great decrease in the absolute amount of plasma protein is found.

The alterations in the percentage and absolute amounts of albumin and globulin paralleled fairly closely the changes in the total protein

Removal of the spleen in some experiments did not seem to affect the results indicating that the spleen was not in the main responsible for the alterations in the proportion of red blood cells to plasma

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# A STUDY OF THE EFFECTS OF HEMORRHAGE, TRAUMA, HISTAMINE AND SPINAL ANESTHESIA ON THE COMPOSITION OF THE BLOOD WHEN NO FLUIDS ARE INJECTED AND WHEN FLUIDS ARE INTRODUCED INTRAVENOUSLY

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In previous studies (1, 2) the composition of the blood has been determined before, during and after the injection of various fluids intravenously under normal and abnormal conditions. The abnormal conditions consisted of some experiments in which a decline in blood pressure was produced by trauma to the intestines and others in which probably due either to the anesthetic or to the fluid that was injected intravenously there was an early marked decline in the pressure. The results of these experiments led us to further studies on the composition of the blood of animals in which a decline in pressure was produced by other methods. These methods consisted of (1) graded hemorrhages, (2) trauma to an extremity, (3) the subcutaneous injection of histamine and (4) spinal anesthesia. These procedures were chosen because a large number of the instances of low blood pressures in patients are due to similar causes. Experiments were performed both with and without the introduction of fluids intravenously. The fluids that were chosen for study were normal salt solution and Evans' gum acacia-glucose-saline solution. These two fluids together with others were employed in the previous studies and in the present experiments they were used as examples of solutions of crystalloids and of colloids.

## METHODS AND RESULTS

The experimental animals in all instances were dogs. They were anesthetized by morphine except in the experiments in which an extremity was traumatized. In the latter experiments, sodium barbital, 0.3 gram per kilogram of body weight, was administered intravenously. The animals gave no evidence of pain and were killed at the completion of the experiments. A cannula that was connected to a mercury manometer was placed in the carotid artery for the blood pressure determinations. Samples of blood for the various analyses were obtained from the femoral vein. The blood that was removed was replaced by an equal volume

At the beginning of all experiments, specimens of blood were removed for determinations of the hemoglobin, the hematocrit, the blood volume, the total protein, albumin and globulin. These studies, except for those on the blood volume, were repeated after intervals of one, two and one-half, four, five and one-half and seven hours. Van Allen tubes were used for the hematocrit determinations. The method of Cohen and Smith (3) was employed in determining the percentage of hemoglobin. Vital red was used in the estimation of the control blood volume. These figures are placed in brackets in the tables. The changes in the blood volume during the course of the experiments were assumed to vary in an inverse ratio to the alterations in the percentage of hemoglobin. The hematocrit readings were used in calculating the volume of red blood cells and plasma. The nitrogen determinations on blood serum and urine were performed by the Kjeldahl-Gunning method. In the tables, nitrogen is expressed as protein. The absolute or entire amounts of total protein, albumin and globulin were obtained by multiplying the percentage of each by the volume of plasma.

A decline in blood pressure was produced by four different methods and for descriptive purposes the experiments are divided into four groups. In each group, studies were first performed on the effects of the procedure when no fluid was introduced intravenously. In subsequent studies, fluids were injected at a constant rate during the first four hours of the experiments. The volume of fluid introduced equalled 10 cc per kilogram of body weight per hour. The fluids injected in the different experiments were normal salt solution and Evans' solution of 6.0 per cent gum acacia and 20 per cent glucose in normal saline. In addition in the experiments on histamine, the effects of injecting blood serum were studied also.

The results of all experiments are summarized in the text. Due to lack of space, the results of only one experiment are given in detail in the tables.

## I EXPERIMENTS ON THE EFFECTS OF GRADED HEMORRHAGE

The entire amount of blood that was withdrawn equalled 4.0 per cent of the body weight. Its removal was distributed as follows. After the control determinations had been performed, a volume of blood equal to 1.0 per cent of the body weight was removed. One hour later and two and one-half hours later just prior to the withdrawal of samples for the analyses, blood equalling 1.5 per cent of the body weight was removed.

Since blood was withdrawn from the circulation, the calculations were somewhat different from those in most of the other experiments. After calculating the alterations in the blood volume from the original by the use of the hemoglobin and hematocrit determinations, deduction was made for the amount of blood that had been removed. The absolute

amount of plasma protein was obtained by multiplying the volume of plasma by the percentage of protein. The sum of this figure and the amount of protein that was present in the blood that was removed is placed in parentheses in the tables

*A The effects of graded hemorrhages alone on the composition of the blood*

Three experiments were performed in which the effects of graded hemorrhage were studied. No fluid was introduced. In two of the experiments, there was a large decline in the arterial pressure. The alterations in the hemoglobin and hematocrit readings were very small and inconstant. There were decreases in the volumes of whole blood, of plasma and of red blood cells. The percentages of total protein, of albumin and of globulin decreased very slightly. The absolute amounts of the protein constituents in the blood plasma decreased. However, if one includes in these figures the amount of protein that was present in the withdrawn blood, it is to be seen in one experiment that there was an increase in the absolute amount of protein constituents, in another there was no alteration and in the third there was a slight decrease. The results of one of these experiments are given in Table I.

*B The effects of graded hemorrhages and of the intravenous injection of normal salt solution on the composition of the blood*

Three such experiments were performed. In two of the three experiments there was a marked decline in the blood pressure. There was a decrease in the hemoglobin and hematocrit readings in all experiments. The volume of plasma declined rather markedly in two of the three experiments. A reduction of the percentages of total protein, albumin and globulin in the blood serum was found. The absolute amounts of the protein constituents in the blood plasma decreased. However, if one adds to that in the blood stream the protein that was present in the blood that was withdrawn, it is to be seen in two of the experiments that there was an increase in the absolute protein. The results of one of these experiments are to be found in Table I.

*C The effects of graded hemorrhages and of the intravenous injection of gum acacia glucose saline solution on the composition of the blood*

Only one experiment of this type was performed. The blood pressure did not decline until after the injection of fluid was stopped. There was a decrease in the percentage of hemoglobin and in the hematocrit readings. The volume of plasma increased during the injection of the fluid and returned later to the original level. There was a marked decrease in the percentages of total protein, albumin and globulin. The absolute amounts of the protein constituents in the blood plasma decreased. The sum of the protein that remained in the plasma of the



Experiment number and weight	Time from beginning	Amount of fluid given	Blood removed	Total protein		Albumin		Globulin		Blood volume			Hemato- crit	Hemo- toblin	Mean blood pressure	
				Serum	I or total serum volume	Serum	I or total serum volume	Serum	per cent	grams	per cent	grams				Red blood cells
No fluid introduced																
1 102 20.0 kgm	Control		0	5.88	72.1 (71.2)†	3.05	37.1 (35.5)†	2.83	31.7 (35.7)†	[832]*	[1225]*	[2057]*	10.4	89.3	137	
	1°		200	5.78	64.1 (69.6)†	2.87	31.9 (35.5)†	2.91	32.2 (31.1)†	726	1108	1834	39.6	90.4	118	
	2° 30'		500	5.60	52.0 (72.1)†	2.87	26.7 (36.4)†	2.73	25.3 (35.7)†	625	928	1553	10.2	89.3	107	
	1°		655	5.60	19.3 (72.5)†	2.83	21.9 (36.9)†	2.77	21.6 (35.6)†	568	880	1118	39.2	86.2	106	
	5° 30'			5.53	19.7 (69.6)†	2.83	25.1 (35.0)†	2.70	21.3 (31.6)†	550	898	1118	38.0	86.0	96	
	7°			5.62	16.8	2.83	23.5	2.79	23.3	568	832	1100	10.6	89.0	90	
Salt solution, 0.9 per cent, introduced intravenously																
1 09 16.1 kgm	Control	0	0	6.64	51.1 (50.9)†	1.06	31.1 (30.7)†	2.58	20.0 (20.2)†	[554]*	[774]*	[1328]*	11.8	93.5	135	
	1°	164	164	6.18	11.6 (51.0)†	3.89	26.8 (32.7)†	2.59	17.8 (21.5)†	469	688	1157	10.5	91.0	135	
	2° 30'	110	110	5.44	38.2 (55.9)†	3.29	23.1 (31.0)†	2.15	15.1 (21.9)†	383	702	1085	35.3	78.1	132	
	1°	656	656	5.18	31.1 (57.4)†	3.16	19.2 (31.5)†	2.02	12.2 (22.9)†	286	606	892	32.0	73.5	125	
	5° 30'			5.92	32.9 (56.3)†	3.54	19.7 (34.5)†	2.38	13.2 (22.8)†	287	556	843	31.0	77.3	116	
	7°			5.66	31.8	3.51	19.7	2.15	12.1	290	562	852	31.0	76.5	101	

TABLE I (continued)

Experiment number and weight	Time from beginning	Amount of fluid given	Blood removed	Total protein		Albumin		Globulin		Blood volume			Hema-tocrit	Hemo-globin	Mean blood pressure
				Serum	For total serum volume	Serum	For total serum volume	Serum	For total serum volume	Red blood cells	Plasma	Whole			
		cc.	cc.	per cent	grams	per cent	grams	per cent	grams	cc.	cc.	cc.	per cent	per cent	mm. Hg
Gum acacia glucose saline solution introduced intravenously															
T 109	Control	0	0	6.35	47.8	4.19	31.5	2.16	16.3	[482]*	[753]*	[1235]*	39.1	91.5	126
127	1°	127	127	4.79	(51.1)†	3.25	(34.5)†	1.54	(16.6)†	411	899	1310	31.5	77.3	128
kgm.					(54.7)†		(37.0)†		(17.7)						
	2° 30'	318	318	3.84	37.5	2.61	25.5	1.23	12.0	389	978	1367	28.5	63.3	130
					(54.6)†		(38.4)†		(16.3)†						
	4	508	508	3.27	30.5	2.35	21.9	.92	8.6	311	934	1245	25.0	59.1	130
					(50.7)†		(38.6)†		(12.1)†						
	5° 30'			3.62	26.4	3.03	(40.2)†	.59	4.3	320	730	1050	30.4	71.1	113
					(53.4)†				(13.3)†						
	7			3.75	29.3	3.03	23.7	.72	5.6	312	783	1095	28.5	68.2	85

\* Determined directly by the dye method

† Indicates the entire amount that would have been present in the blood stream had protein not been present in the fluid that was injected

Protocols Morphine as anesthetic in all experiments.

T 102 Total urine 99 cc. with a total protein equivalent of 8 grams Stomach contained 45 cc. of fluid at completion of experiment

T 99 Total urine 87 cc. with a total protein equivalent of 9.2 grams Stomach contained 20 cc. of fluid with a total protein equivalent of 0.34 gram.

T 109 Total urine 495 cc. with a total protein equivalent of 10.5 grams. No fluid in stomach or peritoneal cavity at completion of experiment.

blood stream and that removed gave a figure in excess of that obtained during the control period. The results of this experiment are given in Table I.

## II. EXPERIMENTS ON THE EFFECTS OF TRAUMA TO AN EXTREMITY

As has been stated, the animals were deeply anesthetized by sodium barbital. After the control determinations had been performed, one of the posterior extremities was traumatized by striking it with a hammer. In some instances, slight additional trauma was subsequently necessary since the blood pressure did not decline sufficiently as a result of the initial injury. At the completion of the experiments, the posterior portion of the body was divided into two parts by a method previously described (4). The difference in the weights of the two parts was considered a measure of the fluid that was lost from the blood stream into the injured area.

The various determinations were performed in these experiments as in all others that are reported in this paper. However, due to the fact that the amount of blood that was lost into the injured area could not be determined during the course of the experiments, some of the calculations were impossible. An attempt was made to calculate the blood volume and the absolute amounts of the protein constituents at the termination of the experiments. It is realized that the method employed is not in any sense absolute. As usual, the control blood volume and the alterations in the hematocrit and hemoglobin readings were used in computing the changes in the volumes of red blood cells, plasma and whole blood. From the blood volume thus obtained, the amount of fluid that was lost into the injured area as determined by the amputations was subtracted and the result was considered as the blood volume at the end of the experiment. The fluid that was lost into the extremity was considered as having an hematocrit which was the average of all those obtained on the venous blood throughout the experiment. With this assumption the volumes of red blood cells and of plasma in the injured area were calculated. These figures were subtracted from the volumes of red blood cells and of plasma which presumably would have been present in the blood stream had not the loss occurred.

### *A. The effects of trauma to an extremity on the composition of the blood*

Two experiments on the effects of trauma to an extremity were performed. The arterial pressure declined markedly in both experiments. There was an increase in the hematocrit and hemoglobin readings. The volumes of whole blood, plasma and red blood cells decreased. The content of the serum in total protein, albumin and globulin was not altered significantly during the experiments. The absolute amount of the protein constituents was greatly diminished. The results of one of these experiments are given in Table II.



TABLE II (continued)

Experiment number and weight	Time from beginning	Amount of fluid given cc	Total protein		Albumin	Globulin	Blood volume				Hemo- toerit	Hemo- globin		Mean blood pressure
			Serum	1 or total serum volume	Serum	For total serum volume	Serum	1 or total serum volume	Red blood cells	Plasma	Whole	per cent	per cent	mm Hg
			per cent	grams	per cent	grams	per cent	grams	cc	cc	cc			
Gum acacia-glucose salt solution introduced intravenously														
1117 112 kgm	Control	0	8.31	71.6	2.10	20.7	5.91	50.9	[113]*	[862]*	[1305]*	31.0	76.5	166
	1°	112	6.25		1.92		1.33					29.7	69.1	155
	2° 30'	355	5.52		1.73		3.79					28.7	65.8	118
	1°	568	1.63		1.50		3.13					26.1	58.8	120
	5° 30'		1.95		1.62		3.33					21.2	61.5	75
	7°		5.00	28.8	1.68	9.7	3.32	19.1	257	576	833	30.0	65.1	59
	Injected blood		7.11		3.13		3.68					39.6	90.0	

\* Determined directly by the dye method

Sodium barbital as anesthetic in all experiments

Protocols T 112 Weight of traumatized extremity 2925 grams Weight of non traumatized extremity 2375 grams Difference in weight 550 grams Total urine during experiment 23 cc Weight of the experiment 2150 grams Difference in weight 550 grams T 131 Slight additional trauma was carried out several times during the first 2 hours of the experiment Weight of opposite non-traumatized extremity 3295 grams Weight of traumatized extremity 2450 grams Difference in weight 1115 grams Total urine 5 cc with a total protein equivalent of 0.1 gram Weight of non traumatized extremity 2450 grams Total urine 300 cc with a total protein equivalent of 111 grams T 117 The mean blood pressure declined to a mean level of 100 mm Hg shortly after the leg was traumatized and it remained there for several minutes Weight of traumatized extremity 2450 grams Weight of non traumatized extremity 1750 grams Difference in weight 700 grams Total urine 300 cc with a total protein equivalent of 111 grams

*B The effects of trauma to an extremity and of the intravenous injection of normal salt solution on the composition of the blood*

Three experiments of this type were performed. A greater amount of trauma was necessary for the production of a low blood pressure than in the experiments in which no fluid was injected. In two of the present experiments, the blood pressure did not reach a low level until after the introduction of fluid had been terminated. The alterations in the hemoglobin and hematocrit readings were small and variable. The volume of blood plasma decreased markedly. There were small but definite decreases in the percentages of total protein, albumin and globulin in the blood serum. The absolute amounts of the protein constituents decreased markedly. The results of one of these experiments are contained in Table II.

*C The effects of trauma to an extremity and of the intravenous injection of gumi acacia-glucose saline solution on the composition of the blood*

Two experiments of this type were performed. The blood pressure declined during the administration of fluid but did not reach a low level until after the injection was stopped. There was a marked decrease in the hemoglobin and hematocrit readings. The calculated volume of plasma increased in one experiment and decreased in the other. There were marked decreases in the percentages of total protein, albumin and globulin in the blood serum. The absolute amounts of the protein constituents decreased. The results of one of these experiments are to be found in Table II.

### III EXPERIMENTS ON THE EFFECTS OF HISTAMINE

The histamine solution that was injected subcutaneously contained one milligram of the drug per cubic centimeter of salt solution. It was given in sufficient amounts to maintain the blood pressure definitely depressed during the first four hours of the experiments.

*A The effects of the subcutaneous injection of histamine on the composition of the blood*

Three experiments were performed in which the effects of the subcutaneous injection of histamine were studied. The blood pressure rose following the completion of the injections but did not return to the previous control level. The concentration of the red blood cells and the percentage of hemoglobin increased in all experiments. The volume of blood plasma diminished. There was a slight increase in the percentages of total protein, albumin and globulin in the blood serum. The absolute amounts of the protein constituents in the blood plasma decreased. The results of one of these experiments are given in Table III.

TABLE III  
The effects of the subcutaneous injection of histamine on the composition of the blood

Experiment number and weight	Time from beginning	Amount of fluid given	Total protein			Albumin			Globulin			Blood volume				Hemo tocrit	Hemo globin	Mean blood pressure
			Serum	per cent	grams	For total serum volume	Serum	per cent	grams	Red blood cells	Plasma	Whole						
												cc	cc					
No fluid introduced																		
189	Control		6.99		51.8	4.62		31.2	2.37	17.6	[555]*	[710]*	[1295]*	12.8	113.6	130		
151	1°		7.75		36.7	1.87		23.0	2.88	13.7	561	173	1037	51.1	111.2	117		
kgm	2° 30'		7.80		36.5	1.73		22.1	3.07	11.1	569	168	1037	51.8	111.2	99		
	1°	53	8.05		36.0	1.98		22.2	3.07	13.8	553	117	1000	55.3	117.1	99		
	5° 30'		7.51		36.2	1.59		22.0	2.95	11.2	560	180	1010	53.9	111.1	88		
	7°		7.15		36.1	1.59		22.2	2.86	13.9	556	181	1010	53.5	111.1	107		
	Injected blood		7.15			2.17			5.28					30.3	80.6			
Salt solution, 0.9 per cent, introduced intravenously																		
195	Control	0	5.60		33.1	3.85		23.0	1.75	10.1	[103]*	[597]*	[1000]*	10.3	96.8	111		
117	1°	117	1.91		25.2	3.30		16.8	1.64	8.1	101	509	913	11.2	106.0	91		
kgm	2° 30'	291	1.58		21.7	3.05		16.5	1.53	8.2	118	540	958	13.6	101.0	75		
	1°	165	1.17		21.0	2.91		15.6	1.56	8.1	113	536	919	11.6	102.0	65		
	5° 30'		4.72		21.8	3.10		11.3	1.62	7.5	101	161	865	16.7	111.9	98		
	7°		1.90		25.0	3.15		16.0	1.75	9.0	101	509	913	15.2	106.3	110		
	Injected blood		6.18			3.68			2.80					30.5	77.7			
Gum acacia glucose-salt solution introduced intravenously																		
108	Control	0	7.06		13.8	1.22		26.2	2.81	17.6	[372]*	[620]*	[992]*	37.1	92.0	135		
139	1°	139	1.51		28.3	2.51		15.7	2.03	12.7	377	623	1000	37.7	91.2	70		
kgm	2° 30'	318	3.25		27.6	1.95		16.6	1.30	11.0	388	852	1210	31.1	73.5	80		
	1°	556	2.78		27.9	1.65		16.5	1.13	11.1	392	1005	1397	28.0	65.2	56		
	5° 30'		3.15		28.8	1.96		18.0	1.19	10.9	387	913	1300	29.8	70.1	16		
	7°		3.59		30.5	2.12		18.1	1.47	12.1	381	852	1233	31.0	73.9	15		
	Injected blood		6.10			3.42			2.68					33.1	76.1			

TABLE III (continued)

Experiment number and weight	Time from beginning	Amount of fluid given	Total protein		Albumin		Globulin		Blood volume			Hemo-globin	Mean blood pressure
			Serum	For total serum volume	Serum	For total serum volume	Serum	For total serum volume	Red blood cells	Plasma	Whole		
		cc.	per cent	grams	per cent	grams	per cent	grams	cc	cc.	cc.	per cent	mm Hg
Blood serum introduced intravenously													
T 107	Control	0	6.01	49.3 (26.6)†	3.64	29.8 (13.1)†	2.37	19.5 (13.5)†	[580]*	[820]*	[1400]*	41.4	86.7
15.5 kgm.	1°	155	7.04	37.1 (25.5)†	3.61	19.0 (11.6)†	3.43	18.1 (13.9)†	559	526	1085	51.4	111.9
	2° 30'	388	6.84	51.8 (24.3)†	3.49	26.5 (9.6)†	3.35	25.3 (14.7)†	585	758	1343	43.5	90.4
	4°	620	6.78	66.4 (27.3)†	3.38	33.1 (10.8)†	3.40	33.3 (16.5)†	565	980	1545	36.6	78.5
	5° 30'		6.68	69.4 (14.7)†	3.30	34.3 (5.8)†	3.38	35.1 (8.9)†	560	1040	1600	35.0	75.8
	7°		6.78	56.8	3.49	29.3	3.29	27.5	572	838	1410	40.5	64
	Injected serum		6.78		3.78		3.00						

\* Determined directly by the dye method

† Indicates the entire amount that would have been present in the blood stream had protein not been present in the fluid that was injected

Protocols. Morphine as anesthetic in all experiments.

T 89 Total urine 68 cc with a total protein equivalent of 9.3 grams.

T 95 Total urine 77 cc. with a total protein equivalent of 11.2 grams. 190 cc. of fluid in stomach with a total protein equivalent of 0.9 gram. Small amount of bloody fluid was passed from rectum.

T 108 Total urine 61 cc. with a total protein equivalent of 0.8 gram. No fluid in stomach at completion of experiment. At autopsy, 150 cc. of fluid which clotted after removal was recovered from the peritoneal cavity. This fluid had a total protein content of 6.25 grams per 100 cc., an albumin content of 2.01 grams and a globulin content of 4.24 grams.

T 107 Total urine 10 cc. with a total protein equivalent of 0.2 gram. Stomach contained 195 cc. of fluid with a total protein equivalent of 3.9 grams.



*B The effects of the subcutaneous injection of histamine and of the intravenous injection of normal salt solution on the composition of the blood*

Three experiments of this type were performed. A marked decline in the blood pressure was produced in all of them. There was a definite but not great increase in the hematocrit and hemoglobin readings in two of the experiments. In the remaining experiment, there was a slight decline. The volume of blood plasma decreased in two experiments and increased slightly in one. There was a decrease in the percentage of the protein constituents of the serum in all experiments. In none of these was the decrease very great. The absolute amounts of the protein constituents decreased in two experiments and remained at approximately the control level in the other one. At the completion of each of the experiments, the stomach contained a large amount of fluid. The results of one of these experiments are to be found in Table III.

*C The effects of the subcutaneous injection of histamine and of the intravenous injection of gum acacia-glucose-saline solution on the composition of the blood*

Two experiments were performed. A marked decline in the blood pressure was produced in both. After a slight initial increase, there was a great decrease in the hemoglobin and hematocrit readings. The volume of blood plasma increased. There was a great decrease in the percentages of total protein, albumin and globulin in the blood serum. The absolute amounts of the protein constituents decreased. The results of one of these experiments are given in Table III.

*D The effects of the subcutaneous injection of histamine and of the intravenous injection of blood serum on the composition of the blood*

Two experiments were performed in which the effects of the injection of histamine and of blood serum were studied. The serum was obtained from a normal dog the blood of which was not incompatible with that of the experimental animal. A marked decline in the blood pressure followed the injection of histamine in each experiment. There was an increase in the hemoglobin and hematocrit determinations during the early part of the experiments and a decrease later. The volume of blood plasma first decreased slightly and then increased. There was an increase in the percentages of total protein and globulin in the blood serum, except for an initial decrease in one experiment. The absolute amounts of the protein constituents in the blood plasma increased greatly. However, if one corrects for the protein that was present in the injected serum, a decrease in the absolute amount of plasma protein is found. The latter figures are placed in brackets in the table. The results of one of these experiments are to be seen in Table III.

## IV EXPERIMENTS ON THE EFFECTS OF SPINAL ANESTHESIA

A five per cent solution of procaine hydrochloride was used as the spinal anesthetic. With the animal lying on its side, the fluid was introduced into the canal in the lumbar region. Small amounts of the procaine were injected frequently until a marked decline in the blood pressure resulted. The animal was then placed on its back.

*A The effects of spinal anesthesia alone on the composition of the blood*

Four experiments are reported in which the effects of spinal anesthesia were studied. In several others, either no significant decline in pressure was produced or death followed shortly after the injection of the procaine. The concentration of the blood and the percentage of hemoglobin increased in three of the four experiments but in no instance was the increase very great. In three of the experiments, there was a slight decrease in the volume of plasma. However, in two of these, the decline did not appear during the first hour of the experiment when the blood pressure was lowest. The percentages of the protein constituents in the blood serum remained practically unaltered during the experiments. In two of the experiments, the absolute amount of plasma protein remained at approximately the control level. In the remaining two, there was no alteration during the first hour, and following this there was a decline. The results of one of these experiments are given in Table IV.

*B The effects of spinal anesthesia and of the intravenous injection of normal salt solution on the composition of the blood*

Two experiments of this type were performed. A marked decline in the blood pressure was produced in each. At the end of the first hour, there was a slight decrease in the hemoglobin and hematocrit readings in each. Following this, they rose to levels a little higher than those observed during the control periods. The plasma volume first increased and then decreased slightly. There were small decreases in the percentages of total protein, albumin and globulin in the blood serum. The absolute amounts of the protein constituents declined but not to a great extent. The results of one of these experiments are contained in Table IV.

*C The effects of spinal anesthesia and of the intravenous injection of gum acacia glucose-saline solution on the composition of the blood*

Two experiments were performed. In one of these, the blood pressure remained markedly depressed, while in the other it returned to the control level and remained there several hours. In one experiment the concentration of the red blood cells decreased during the injection of the fluid, and in the other the initial increase in the concentration was followed by dilution. The hematocrit and hemoglobin readings increased following the termination of the injections. There was a decrease in the percent-

TABLE IV  
*The effects of spinal anesthesia on the composition of the blood*

Experi- ment number and weight	Time from beginning	Amount of fluid given	Total protein		Albumin		Globulin		Blood volume			Hemo- globin	Mean blood pressure
			Serum	For total serum volume	Serum	For total serum volume	Red blood cells	Plasma	Whole				
										per cent	grams		
No fluid introduced													
T 131 20 0 kgm	Control		6 86	82 0	2 89	34 5	3 97	47 5	[716]*	[1194]*	[1910]*	37 5	150
	1°		6 81	83 8	2 85	35 0	3 96	48 8	725	1230	1955	37 0	70
	2° 30'		6 97	79 2	2 92	33 2	4 05	46 0	733	1137	1870	39 1	138
	4°		6 86	78 2	2 89	33 0	3 97	45 2	738	1142	1880	39 2	130
	5° 30'		6 86	73 7	2 89	31 1	3 97	42 6	725	1075	1800	40 3	118
	7° Injected blood		6 64	72 1	3 03	32 8	3 61	39 3	715	1085	1800	39 7	116
			5 07		2 78		2 29					22 9	51 0
Salt solution, 0.9 per cent, introduced intravenously													
T 133 16 5 kgm	Control	0	6 77	76 3	3 29	37 1	3 48	39 2	[593]*	[1127]*	[1720]*	34 4	124
	1°	165	6 33	80 7	3 08	39 3	3 25	41 1	575	1275	1850	31 0	80
	2° 30'	412	6 12	66 4	3 02	32 8	3 10	33 6	600	1085	1685	35 6	150
	4°	660	5 98	67 3	2 97	33 5	3 01	33 8	595	1125	1720	34 5	118
	5° 30'		6 37	70 0	3 04	33 3	3 33	36 7	608	1097	1705	35 7	102
	7°				3 00	32 1			595	1070	1665	35 7	100

TABLE IV (continued)

Experi- ment number and weight	Time from beginning	Amount of fluid given	Total protein			Albumin		Globulin		Blood volume				Hemo- globin  per cent	Mean blood pressure
			Serum	For total serum volume	Serum	For total serum volume	Serum	For total serum volume	Red blood cells	Plasma	Whole				
		cc.	per cent	grams	per cent	grams	per cent	grams	cc.	cc.	cc.	per cent	per cent	mm Hg	
Gum acacia glucose-salt solution introduced intravenously															
T 130	Control	0	7.64	61.8	3.04	24.6	4.60	37.2	[454]*	[808]*	[1262]*	36.0	89.0	125	
156	1°	156	5.38	48.1	2.05	18.3	3.33	29.8	461	894	1355	34.0	82.9	56	
kgm	2° 30'	390	4.94	44.2	1.89	17.0	3.05	27.3	466	896	1362	34.2	82.4	144	
	4°	624	4.16	43.6	1.56	16.3	2.60	27.3	448	1048	1496	30.0	75.0	132	
	5° 30'		4.90	40.2	1.97	16.2	2.93	24.1	489	821	1310	37.3	85.7	120	
	7°		4.98	40.5	2.05	16.7	2.93	23.8	511	814	1325	38.6	84.7	94	
	Injected blood		6.63		2.71	3.92						28.5	67.9		

\* Determined directly by the dye method

Protocols. Morphine as narcotic in all experiments

T 131 Total urine 210 cc. with a total protein equivalent of 1.9 gram

T 133 Given 5.0 cc. of 5.0 per cent procaine hydrochloride over a period of one hour. Decline in blood pressure to 30 mm Hg at completion of injection. Total urine 60 cc. with a total protein equivalent of 4.4 grams.

T 130 Procaine 1.6 cc. Total urine 250 cc. with a total protein equivalent of 8.9 grams.

ages of the total protein, albumin and globulin in the blood serum. The absolute amounts of the protein constituents in the blood plasma decreased. The results of one of these experiments are given in Table IV.

### DISCUSSION

In a previous paper (2) experiments were reported in which the cause for the decline in blood pressure was not clearly understood, and in which the injection of fluids intravenously was usually associated with marked decreases in both the percentage and absolute amount of plasma protein. In view of these findings it seemed important to determine whether or not a decline in blood pressure produced in other ways would be associated with similar alterations. For example, if the intravenous injection of salt solution following a severe hemorrhage were associated with a marked decrease in the percentage and absolute amount of protein in the blood serum, its use might be harmful rather than beneficial. The four methods by which the decline in pressure was produced were arbitrarily chosen as being fairly representative of the different ways of causing such a condition.

The findings in the experiments on the effects of hemorrhage were quite different from those previously reported (2) in which the unexplained early decline in the pressure took place. When normal salt solution was injected into the animals from which blood was being removed, a decrease rather than an increase in the concentration of the red blood cells was found. The percentage of protein in the serum decreased but when the protein that was removed was added to that remaining in the blood stream, it was found that the absolute amount of plasma protein usually increased. Calculated in this manner, the increase in one experiment was five grams and in another six grams. In the experiments on hemorrhage the blood pressure readings as listed in the tables are not in all instances very low but they are somewhat misleading in this respect in that the pressure that existed just before the removal of the blood was the one used. During and shortly following the removal of blood, the blood pressure was frequently markedly depressed. The decline in pressure usually appeared much earlier in the experiments with the unexplained fall than in those in which blood was removed. It is possible that the vasoconstriction of the vessels which usually accompanies hemorrhage was responsible for the prevention of the loss of protein through the vessel walls in the present experiments, but we have no proof of this.

The addition of the protein that was removed to that remaining in the blood stream in the experiments on hemorrhage indicated an increase in the absolute amount of plasma protein in most of the experiments. Scott (5) from his studies on the mechanism of the absorption of fluid from the tissue spaces states "The above results show that as large or larger increases in the nitrogenous bodies of the plasma occur after injecting

Ringer's fluid (when all backward filtration is excluded) as occur after hemorrhage. After hemorrhage, the fluid passing back into the blood must contain less protein than the plasma whether it passes back by osmosis or by filtration, it will then necessarily cause protein to pass from the cells into the plasma. Consequently an increase of protein in the plasma after hemorrhage is no evidence of backward filtration and is consistent with Starling's view that the fluid passes into the capillaries by osmotic action." In previous experiments in which the effects of the injection of solutions of crystalloids into normal dogs were determined, there was a slight increase in the absolute amount of the plasma protein in several of the experiments but in most of them it remained at the same level or decreased slightly. However, in the present experiments in which both the effects of hemorrhages and the injection of fluids were studied, there was usually an increase in the plasma protein. If Scott is correct in his statement that backward filtration is excluded when fluid that has been injected is passing through the vessels, then it follows that protein did not pass from the tissue spaces into the blood stream in our experiments because part of the fluid that was injected escaped through the vessel walls. However, it is not necessarily true that backward filtration is excluded simply because part of the fluid that was injected was leaving the blood stream. It would seem to be possible that at any given time fluid could be leaving the blood stream in some areas while at the same time fluid could be passing into the circulation in other areas.

The experiments on the effects of trauma to an extremity differ in the main from those on hemorrhage in that the latter type was usually associated with a decrease in the concentration of the red blood cells, whereas a slight increase usually occurred in the former. The difference is probably due to the fact that there was a greater proportional loss of plasma than of red cells in the studies on trauma whereas hemorrhage resulted in the loss of blood as it existed at the time in the blood vessels. The above remarks do not apply to the experiments in which the gum acacia solution was injected, since in both types a marked decrease in the concentration of the red blood cells was found. The increase in the concentration of the blood in the experiments on the effects of trauma and the introduction of salt solution was not nearly so marked as in the previous experiments (2), in which the unexplained declines in pressure occurred. Also, the decrease in the percentage of protein in the blood serum in the present experiments was not nearly so great as in the former ones. Unfortunately the absolute amounts of the protein constituents could not be determined accurately during the course of the experiments on trauma. Since the fluid that escaped from the blood stream into the injured area had approximately the same composition as whole blood and since there was such a marked difference in the weight of the traumatized

and non-traumatized parts, it is likely that most if not all of the protein was lost into the damaged tissues. The impression was gained that it took a greater amount of trauma to produce a decline in blood pressure when salt solution was injected than when no solutions were given. This observation would indicate that the salt solution was not exerting harmful effects.

Protein was usually lost from the blood stream when histamine and fluids were injected but it was surprising to us that the loss was not greater. The reduction in the percentage and absolute amount of plasma protein was usually not as great in these experiments as in the previous (2) ones with the unexplained decline in pressure. In the latter experiments the decline in pressure was frequently maintained for only a short while, whereas in the experiments on histamine the blood pressure was kept at a low level for a long time. At the completion of most of the experiments on histamine, the stomach contained large amounts of fluid. The content of this fluid in protein was quite low. It was sufficient to account for only a small part of the total loss of protein.

In the experiments on the effects of spinal anesthesia alone, there was either no loss in the absolute amount of plasma protein or the loss was not very great. When normal salt solution was introduced intravenously after producing a decline in pressure by spinal anesthesia, there was not a large loss of protein. The decline in the blood pressure in these experiments was similar to that observed in the experiments (2) in which the unexplained fall occurred. In both instances, a marked drop in the pressure was noted during the early part of the studies. It is possible that the absence of a large loss of protein in the experiments on spinal anesthesia was due to the fact that part rather than the whole of the body was affected by the anesthetic. In the experiments in which the unexplained decline in the pressure occurred and in those in which histamine was injected, the effects were probably general rather than local.

It is noted in some of the experiments reported in this paper that it is possible to have a marked decline in the blood pressure without an associated diminution in the absolute amount of plasma protein. This finding indicates that the unexplained decline in blood pressure in the experiments previously reported (2) was not entirely responsible for the loss.

### SUMMARY

The effects on the composition of the blood of four different procedures which resulted in a decline in the blood pressure were determined in some dogs in which no fluid was injected and in others in which either normal salt solution or gum acacia-glucose-saline solution was introduced intravenously. The methods that were employed in reducing the blood pressure consisted of (1) the graded removal of blood, (2) trauma to an extremity, (3) the subcutaneous injection of histamine and (4) the injection

of procaine hydrochloride into the spinal canal. The studies included determinations of the carotid blood pressure, the percentage of hemoglobin, the concentration of the red blood cells, the volumes of whole blood, red blood cells and plasma, and the percentages of total protein, albumin and globulin in the blood serum. The absolute amounts of the protein constituents were calculated by multiplying the percentage of each in the serum by the volume of plasma.

Some of the results that were obtained are as follows

1 In the experiments on the effects of hemorrhage, both with and without the introduction of fluids, the amount of protein that was present in the blood that was removed was usually more than sufficient to account for the decrease in the total amount of protein in the plasma of the blood stream. The results of most of the experiments indicate that protein passed into the blood vessels.

2 In both the experiments on hemorrhage and those on trauma to an extremity, there was a slight decrease in the concentration of the plasma protein associated with the introduction of normal salt solution. The total amount of protein that was lost from the blood stream in the experiments on trauma could not be determined with accuracy but the results indicate that most if not all of it escaped into the injured area.

3 In all of the experiments with one exception in which histamine was injected, there was a decrease in the absolute amounts of the protein constituents. However, the loss was not as great as was usually encountered in the previous experiments (2) in which the unexplained decline in blood pressure occurred.

4 There was a comparatively small loss of protein from the blood stream in the experiments in which a marked decline in the blood pressure followed the injection of procaine into the spinal canal. The findings were similar when in addition normal salt solution was introduced intravenously.

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# THE EFFECTS ON THE COMPOSITION OF THE BLOOD OF THE SUBCUTANEOUS INJECTION OF NORMAL SALT SOLUTION INTO NORMAL DOGS AND INTO DOGS SUBJECTED TO INTESTINAL TRAUMA, GRADED HEMORRHAGES AND HISTAMINE INJECTION

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In previous studies (1, 2, 3), the effects on the composition of the blood of the introduction of fluids intravenously have been determined on normal animals and on animals in which a decline in blood pressure had been produced by a variety of means. Another method frequently employed by which fluids may be introduced consists of injection into the subcutaneous tissues. For this purpose, normal salt solution is used most often. The present studies were undertaken in order to determine the effects on the composition of the blood of the subcutaneous injection of normal salt solution into normal dogs and into dogs in which a decline in blood pressure was produced by several different methods. Also we were interested in determining how much of the fluid that was placed in the tissues was absorbed into the general circulation. For this reason all of the fluid was injected into the tissues of one posterior extremity, groin and flank. At the completion of the experiments, the difference in the weights of the two posterior portions of the body was determined.

## METHODS

Dogs were used in all experiments. Morphine sulphate was employed as an anesthetic in all experiments except those in which the intestines were traumatized. Sodium barbital was used in these. The animals gave no evidence of pain during the course of the experiments. The blood pressure was determined by placing a cannula that was connected to a mercury manometer into the carotid artery. Specimens of blood for the various analyses were obtained from the femoral vein of the extremity into which no fluid was injected and this blood was replaced by an equal amount obtained from a normal dog.

Four different types of experiments were performed. In all of these, following the replacement of the blood which was removed in order to determine the blood volume, hemoglobin, hematocrit, total protein, albumin and globulin, normal salt solution was injected continuously into the tissues of one of the posterior extremities, groin and flank. It was given at body temperature at the rate of 10 cc. per kilogram of body weight per hour for four hours. Samples of blood for the various analyses were obtained one and two and one-half hours

following the beginning of the injection and at its completion. Further samples were obtained after intervals of one and one-half and three hours. The animals were then killed and the difference in the weights of the two posterior portions of the body was determined. In the first group of experiments, the effects on the composition of the blood of the introduction of fluids into the subcutaneous tissues of normal animals were studied. In the second group, after making a midline abdominal incision, the intestines were traumatized during the four hours while fluid was being injected by gently passing them between the fingers. At the end of this time, the incision was closed, and two further series of determinations were performed during the following three hours. In the third group of experiments, the effects of graded hemorrhages at the same time that fluid was being introduced subcutaneously were studied. As in all experiments normal salt solution was injected at the rate of 10 cc per kilogram of body weight per hour for four hours and samples of blood were obtained at the usual times. After performing the control determinations, whole blood which equalled one per cent of the body weight was removed from the femoral artery. Blood equalling approximately one and one-half per cent of the body weight was withdrawn one hour later and two and one-half hours later. The volume of the blood that was removed was slightly less than the volume of salt solution that was introduced. The observations were continued for three hours after the completion of the injection of salt solution. In the fourth group of experiments, histamine was injected intermittently into the subcutaneous tissues during the four hours that salt solution was being introduced. It was given in amounts sufficiently large to produce a definite decline in the blood pressure. The usual determinations were performed during the three hours following the termination of the injections.

Van Allen tubes were used in the hematocrit determinations. Hemoglobin estimations were performed by the method of Cohen and Smith (4). The control blood volume was determined by the dye method as employed by Rowntree, Brown and Roth (5). These figures are placed in brackets in the tables. During the course of the experiments, excepting those on hemorrhage, the alterations in the total blood volume were assumed to vary in an inverse ratio to the changes in the percentage of hemoglobin. The volumes of red blood cells and of plasma were calculated from the hematocrit readings. In the experiments in which graded hemorrhages were performed, the calculations were different in that after determining the volume by the method described above, subtraction was made for the amount removed. The determinations of the nitrogen were performed on blood serum. Albumin and globulin were separated by the use of 22.2 per cent sodium sulphate as recommended by Howe (6). The Gunning (7) modification of the Kjeldahl method was employed for determining the albumin and total protein nitrogen of the serum. The total nitrogen of the urine was also determined by this method. In all the tables the nitrogen is expressed as protein. The figures for the entire or absolute amounts of protein were obtained by multiplying the percentage of protein per unit volume of serum by the total amount of plasma in the blood stream. In the experiments on hemorrhage, the figures in brackets represent the addition of the total protein, albumin and globulin that were removed at the time of the bleedings to the calculated absolute amounts of each that remained in the blood stream. Analyses were performed on the blood that was injected in order to replace that removed for the various determinations. The differences between the blood removed and that injected were ignored in the calculations. This introduced very little error.

The method (8) by which the posterior part of the body was divided into two parts was as follows. An abdominal incision was made in the midline line. The symphysis pubis was divided with a saw. The bladder and rectum were removed. The abdominal aorta and vena cava were doubly ligated and divided. The iliac vessels were clamped. A transverse abdominal incision was made at approximately the level of the umbilicus. This was extended through the vertebral column and the front part of the body was discarded. Using a knife and a saw, the structures on either side of the vertebral column of the posterior part were divided in a longitudinal direction. This resulted in a separation of the spinal column and tail from the two posterior portions of the body. The difference in the weight of the part into which fluid had been injected and the opposite part was determined.

## RESULTS

### *1 The effects of the subcutaneous injection of normal salt solution*

Three experiments were performed in which the effects on the composition of the blood of the subcutaneous injection of normal salt solution were determined. The blood pressure remained at approximately the control level in all of the experiments. There was usually a slight diminution in the concentration of red blood cells, and in the percentage of hemoglobin. There was a slight but definite increase in the volume of plasma in all experiments. The alterations in the percentages of total protein, albumin and globulin in the blood serum were very minor. There was an increase in the absolute amounts of total protein, albumin and globulin in the blood plasma in all experiments. Determinations of the difference in the weights of the two posterior extremities in the three experiments indicated that 29 per cent of the fluid that was injected was absorbed into the general circulation. The results of these experiments are given in Table I.

### *2 The effects of continuous trauma to the intestines and of the subcutaneous injection of normal salt solution*

Continuous trauma to the intestines and the subcutaneous injection of normal salt solution were associated with varying degrees of decline in the blood pressure. There was an increase in the concentration of the red blood cells and an increase in the percentage of hemoglobin. The volume of blood plasma decreased in all experiments. The percentages of total protein, albumin and globulin in the blood serum remained at approximately the control levels throughout the experiments. There were rather marked decreases in the absolute amounts of the protein constituents in all experiments. Comparison of the weights of the posterior extremities indicated that 17 per cent of the fluid that was injected in the three experiments was absorbed. The results of these experiments are enumerated in Table II.

TABLE I  
*The effects of the subcutaneous injection of normal salt solution on the composition of the blood*

Experiment number and weight	Time from beginning	Fluid given	Total protein		Albumin		Globulin		Blood volume			Hema- to- crit	Hemo- globin	Mean blood pressure
			Serum	For total serum volume	Serum	For total serum volume	Serum	For total serum volume	Red blood cells	Plasma	Whole			
		cc	per cent	grams	per cent	grams	per cent	grams	cc.	cc	cc	per cent	per cent	mm Hg
T 79 16.9 kgm	Control	0	5.22	59.0	2.67	30.2	2.55	28.8	[630]*	[1130]*	[1760]*	35.8	90.5	116
	1°	169	5.22	60.6	2.74	31.8	2.48	28.8	660	1160	1820	36.6	88.2	100
	2° 30'	422	5.32	62.7	2.81	33.2	2.41	29.5	640	1180	1820	35.0	88.2	122
	4°	676	5.13	66.2	2.73	35.2	2.40	31.0	670	1290	1960	33.8	81.5	126
	5° 30'		5.04	64.1	2.62	33.2	2.42	30.9	690	1270	1960	34.8	81.4	114
	7°		4.95	64.4	2.59	33.6	2.36	30.8	700	1300	2000	34.8	79.8	113
	Injected blood		5.67		2.56		3.11					26.1	65.5	
T 80 18.7 kgm	Control	0	6.18	75.2	3.53	42.9	2.65	32.3	[750]*	[1215]*	[1965]*	38.1	86.7	124
	1°	187	6.22	80.6	3.60	46.7	2.62	33.9	724	1296	2020	35.9	84.2	122
	2° 30'	467	6.53	82.3	3.75	47.3	2.78	35.0	705	1260	1965	36.5	86.7	129
	4°	748	6.30	83.2	3.50	46.2	2.80	37.0	740	1320	2060	35.9	82.7	120
	5° 30'		6.34	87.0	3.87	53.1	2.47	34.9	730	1370	2100	34.8	81.1	120
	7°		6.12	78.8	3.43	44.0	2.69	34.8	715	1285	2000	36.7	85.2	120
	Injected blood		4.97		3.56		2.41					33.6	76.9	

TABLE I (continued)

Experiment number and weight	Time from beginning	Fluid given	Total protein		Albumin		Globulin		Blood volume			Hema tocrit	Hemo- globin	Mean blood pressure
			Serum	For total serum volume	Serum	For total serum volume	Serum	For total serum volume	Red blood cells	Plasma	Whole			
		cc	per cent	grams	per cent	grams	per cent	grams	cc	cc	cc	per cent	per cent	mm. Hg
T 81	Control	0	7.20	66.6	4.82	44.6	2.38	22.0	[724]*	[926]*	[1650]*	44.0	100.0	127
21.6	1°	216	7.15	71.5	4.73	47.3	2.42	24.2	715	1000	1715	41.7	96.1	130
kgm.	2° 30'	540	6.93	67.0	4.59	44.3	2.34	22.7	729	966	1695	43.0	97.4	135
	4°	864	6.79	68.4	4.49	45.3	2.30	23.1	724	1008	1732	41.8	95.2	128
	5° 30'		6.70	72.4	4.32	46.6	2.38	25.8	735	1080	1815	40.6	90.9	118
	7°		6.25	73.4	4.25	50.0	2.00	23.4	740	1175	1915	38.5	86.2	120
	Injected blood		6.08		3.46		2.62					37.0	80.2	

\* Determined directly by the dye method

Protocols. Morphine as anesthetic in all experiments.

T 79 Weight of extremity into which fluid was injected was 2540 grams. Weight of opposite extremity was 2020 grams. Difference in weight 520 grams. Total fluid injected was 676 cc. Amount of fluid absorbed was approximately 156 cc. Total urine 105 cc. with a total protein equivalent of 4.7 grams.

T 80 Weight of extremity into which fluid was injected was 2880 grams. Weight of opposite extremity was 2325 grams. Difference in weight 555 grams. Total fluid injected was 748 grams. Amount of fluid absorbed was approximately 193 cc. Total urine 112 cc. with a total protein equivalent of 17 grams.

T 81 Weight of extremity into which fluid was injected was 3405 grams. Weight of opposite extremity was 2840 grams. Difference in weight 565 grams. Total fluid injected was 864 cc. Amount of fluid absorbed was approximately 299 cc. Total urine 85 cc. with a total protein equivalent of 12.3 grams

TABLE II

*The effects of trauma to the intestines and the subcutaneous injection of normal salt solution on the composition of the blood*

Experiment number and weight	Time from beginning	Fluid given	Total protein			Albumin			Globulin			Blood volume			Hema-tocrit	Hemo-globin	Mean blood pres-sure
			Serum	For total serum volume	Fluid	Serum	For total serum volume	Fluid	Serum	For total serum volume	Fluid	Red blood cells	Plasma	Whole			
			per cent	grams	per cent	per cent	grams	per cent	per cent	grams	per cent	cc.	cc.	cc.	per cent	per cent	mm Hg
T 85 20.3 kgm	Control	0	7.08	77.4	6.80	4.33	47.3	4.75	2.75	30.1	2.05	[871]*	[1094]*	[1965]*	44.3	112.7	154
	1°	203	7.11	63.0	6.80	4.33	38.4	4.75	2.78	24.6	2.05	858	886	1744	49.2	127.0	128
	2° 30'	507	7.20	58.2	5.90	4.29	34.7	4.01	2.91	23.5	1.89	876	808	1684	52.0	131.5	117
	4°	812	7.46	43.0	5.48	4.50	25.9	3.73	2.96	17.1	1.75	840	576	1416	59.4	156.2	117
	5° 30'		7.46	39.7	5.48	4.50	23.9	3.59	2.96	15.8	1.89	813	532	1345	60.4	164.8	38
	Injected blood		6.72			4.34			2.38						36.2	91.5	
T 86 21.3 kgm	Control	0	7.96	69.4	7.98	3.63	31.7	4.75	4.33	37.7	3.23	[694]*	[872]*	[1556]*	44.0	108.7	130
	1°	213	7.46	57.0	6.98	3.20	24.4	4.75	4.26	32.6	3.23	733	733	1466	48.0	115.4	87
	2° 30'	533	7.88	55.8	6.98	3.49	24.7	4.03	4.39	31.1	2.95	722	708	1430	50.5	119.0	76
	4°	852	8.00	58.2	6.66	3.67	26.7	3.87	4.33	31.5	2.79	703	727	1430	49.2	119.0	66
	5° 30'		7.88	54.2	6.46	3.53	24.3	3.60	4.35	29.9	2.86	722	688	1410	51.2	120.0	68
	7°		6.78	52.6	6.02	3.03	23.5	3.50	3.75	29.1	2.52	747	776	1523	49.0	111.9	54
	Injected blood		6.12			3.63			2.49						29.9	71.8	

TABLE II (continued)

Experiment number and weight	Time from beginning	Total protein				Albumin			Globulin			Blood volume				Hemo- tocrit	Hemo- globin	Mean blood pressure
		Serum		Fluid		Serum	Fluid	For total serum volume	Serum	Fluid	For total serum volume	Red blood cells	Plasma	Whole				
		per cent	grams	per cent	grams										per cent			
T 87 12.5 kgm.	Control	cc.	0															
	1°		6.25	39.0		5.87	3.90	24.3	2.35									
	2 30'		6.40	29.7		5.87	4.20	19.5	4.26	1.61								
	4°		6.59	27.0		5.56	4.16	17.1	3.73	1.83								
	5° 30'		6.40	26.5		5.56	4.00	16.6	2.40									
	7		6.03	24.4		5.18	3.73	15.1	3.59	1.59								
	Injected blood		5.87	25.5		4.91	3.69	16.0	3.87	1.04								
			6.30				3.54		2.76									

\* Determined directly by the dye method

## Protocols.

Sodium barbital as anesthetic in all experiments.

- T 85 Weight of extremity into which fluid was injected 3230 grams. Weight of opposite extremity 2635 grams. Difference in weight 595 grams. Total fluid injected 812 cc. Amount of fluid absorbed approximately 217 cc. Total urine 97 cc. with a total protein equivalent of 13.8 grams. The loss of fluid from the peritoneum was probably greater after the trauma was stopped than before. The intestines were very black in color at end of experiment.
- T 86 Weight of extremity into which fluid was injected 3800 grams. Weight of opposite extremity 3050 grams. Difference in weight 750 grams. Total fluid injected 852 cc. Amount of fluid absorbed approximately 102 cc. Total urine 14 cc. with a total protein equivalent of 0.7 gram.
- T 87 Weight of extremity into which fluid was injected 2105 grams. Weight of opposite extremity 1645 grams. Difference in weight 460 grams. Total fluid injected 500 cc. Amount of fluid absorbed approximately 40 cc. Total urine 32 cc.



TABLE III

*The effects of hemorrhage and of the subcutaneous injection of salt solution on the composition of the blood*

Experiment number and weight	Time from beginning	Fluid given cc	Blood removed cc	Total protein		Albumin		Globulin		Blood volume			Hematocrit per cent	Hemoglobin per cent	Mean blood pressure mm Hg
				Serum per cent	For total serum protein grams	Serum per cent	For total serum protein grams	Serum per cent	For total serum protein grams	Red blood cells cc	Plasma cc	Whole cc.			
T 82 140 kgm	Control	0	0	5.85	50.1 (55.8)†	3.20	27.4 (27.9)†	2.65	22.7 (27.9)†	[724]*	[856]*	[1580]*	45.3	109.0	138
	1°	140	140	6.18	51.4 (56.3)†	3.07	25.5 (28.0)†	3.11	25.9 (28.4)†	608	832	1440	43.3	107.0	100
	2° 30'	350	350	6.00	47.0 (57.3)†	2.95	23.1 (28.3)†	3.05	23.9 (29.0)†	567	783	1350	42.0	103.0	100
	4°	560	420	5.68	39.6 (58.1)†	2.85	19.8 (29.2)†	2.83	19.8 (28.9)†	478	697	1175	40.7	101.0	113
	5° 30'			5.45	39.2 (58.1)†	2.73	19.6 (29.2)†	2.72	19.6 (28.9)†	457	718	1175	39.0	95.0	110
	7°			5.45	39.2	2.73	19.6	2.72	19.6	467	718	1185	39.4	94.3	98
	Control	0	0	5.81	49.3 (50.7)†	3.69	31.3	2.12	18.0	[882]*	[848]*	[1730]*	51.0	120.0	136
T 83 164 kgm.	1°	164	164	5.94	46.0 (54.1)†					792	774	1566	51.0	119.0	126
	2° 30'	410	410	6.35	42.3 (52.5)†	3.88	25.8	2.47	16.5	669	665	1334	50.5	119.0	120
	4°	656	636	5.65	33.5 (53.0)†	3.58	21.2	2.07	12.3	516	592	1108	48.2	115.4	114
	5° 30'			5.46	34.0 (53.2)†	3.36	20.9	1.20	13.3	553	622	1175	47.1	112.0	108
	7°			5.45	34.2	3.35	21.0	2.10	13.2	568	628	1196	47.6	110.0	100

TABLE III (continued)

Experiment number and weight	Time from beginning	Fluid given cc.	Blood removed cc.	Total protein		Albumin		Globulin		Blood volume			Hemo-tocrit	Hemo-globin per cent	Mean blood pressure mm. Hg
				Serum per cent	For total serum protein grams	Serum per cent	For total serum protein grams	Serum per cent	For total serum protein grams	Red blood cells cc.	Plasma cc.	Whole cc.			
T 84 160 lgm	Control	0	0	6.08	58.2 (61.5)†	3.62	34.6 (36.6)†	2.46	23.6 (24.9)†	[617]*	[958]*	[1575]*	39.2	93.1	143
	1°	160	160	6.01	55.6 (63.9)†	3.58	33.1 (38.6)†	2.43	22.4 (25.3)†	490	925	1415	37.3	89.3	124
	2° 30'	400	400	5.67	49.0 (63.6)†	3.44	29.7 (38.7)†	2.23	19.3 (24.9)†	371	864	1235	34.8	83.3	80
	4°	640	550	5.47	43.1 (62.8)†	3.37	26.5 (38.1)†	2.10	16.6 (24.7)†	387	788	1175	32.9	78.1	120
	5° 30'			5.54	42.3 (65.1)†	3.37	25.8 (40.5)†	2.17	16.5 (24.6)†	391	764	1155	33.9	79.4	120
	7°			5.60	44.6	3.55	28.2	2.05	16.4	390	796	1186	33.0	77.3	123

\* Determined directly by the dye method

† Indicates the entire amount that would have been present in the blood stream had protein not been present in the fluid that was injected

Protocols. Morphine as anesthetic in all experiments.

T 82 Weight of extremity into which fluid was injected 2335 grams. Weight of opposite extremity 1960 grams. Difference in weight 375 grams. Total fluid injected 560 cc. Fluid absorbed was approximately 185 cc. Total urine during experiment was 28 cc.

T 83 Weight of extremity into which fluid was injected 2750 grams. Weight of opposite extremity 2245 grams. Difference in weight 505 grams. Total fluid injected 656 cc. Fluid absorbed was approximately 151 cc. Total urine 80 cc. with a total protein equivalent of 7.2 grams.

T 84. Weight of extremity into which fluid was injected 2490 grams. Weight of opposite extremity 1980 grams. Difference in weight 510 grams. Total fluid injected 640 cc. Fluid absorbed was approximately 130 cc. Total urine 110 cc.

### *3 The effects of graded hemorrhages and of the subcutaneous injection of normal salt solution*

In the three experiments in which the effects of graded hemorrhages and the subcutaneous injection of salt solution were studied, the removal of blood was sufficient to cause a definite decline in the blood pressure. There was a decrease in the hematocrit readings and in the percentage of hemoglobin in all experiments. The volumes of whole blood, plasma, and red blood cells decreased when the amount of blood that was removed is taken into consideration. There was a slight decrease in the percentages of total protein, albumin and globulin in the blood serum. The absolute amounts of the protein constituents that remained in the blood plasma decreased. However, if one adds to that remaining in the blood stream the amount corresponding to the protein removed by bleeding, it is to be noted that protein probably passed into the vessels during the course of the experiments. These figures are placed in brackets in the tables. Approximately 25 per cent of the fluid that was injected into the extremities was absorbed. The results of these experiments are given in Table III.

### *4 The effects of the subcutaneous injection of histamine and of normal salt solution*

A marked decline in the blood pressure was produced in the three experiments in which the effects of the subcutaneous injection of histamine and salt solution were studied. The blood pressure rose after the injections were terminated. There were marked increases in the concentration of the red blood cells and in the percentage of hemoglobin. There was a rather large diminution in the volume of plasma in the blood stream. The content of the blood serum in total protein, albumin and globulin altered very little. However, due to the loss of plasma, there was a great decrease in the absolute amounts of total protein, albumin and globulin. The difference in weight of the posterior extremities in the three experiments indicated that approximately 22 per cent of the fluid that was injected was absorbed. The results of these experiments are given in Table IV.

## DISCUSSION

The significant alterations that accompanied the subcutaneous injection of salt solution into normal dogs consisted of a slight increase in the volume of plasma and in the absolute amount of plasma protein. The findings differ in the main from those previously reported (2) in which the fluid was given intravenously to normal dogs in that an appreciable decrease in the percentage of protein in the blood serum was not encountered in the present experiments. Less than one-third of the fluid that

TABLE IV

*The effects of the subcutaneous injection of histamine and of normal salt solution on the composition of the blood*

Experiment number and weight	Time from beginning	Fluid given	Total histamine mgm.	Total protein		Albumin		Globulin		Blood volume			Hemato- crit	Hemo- globin	Mean blood pressure
				Serum	For total serum volume	Serum	For total serum volume	Serum	For total serum volume	Red blood cells	Plasma	Whole			
T 91 13.3 kgm	Control	cc.		per cent	grams	per cent	grams	per cent	grams	cc.	cc.	cc.	per cent	per cent	mm. Hg
	1°	133	0	6.62	48.9	4.31	31.8	2.31	17.1	[628]*	[738]*	[1366]*	46.0	121.0	118
	2° 30'	332	30			4.10	26.2			660	640	1300	50.8	127.0	110
	4°	532	40	6.94	32.4	4.48	20.9	2.50	11.5	657	467	1124	58.4	147.1	74
	5° 30'		55	6.39	27.9	4.28	18.7	2.11	8.2	644	436	1080	59.7	153.0	80
	7°			6.39	29.8	4.10	19.5	2.29	10.7	657	467	1124	58.4	147.1	95
T 93 13.7 kgm	Injected blood			6.30	33.4	4.05	21.5	2.25	11.9	693	531	1224	56.6	135.1	95
				6.93		3.31		3.62					32.0		
	Control	0	0	6.24	48.1	3.75	28.9	2.49	19.2	[541]*	[771]*	[1312]*	41.3	109.1	150
	1°	137	20	5.62	28.9	3.53	18.2	2.09	10.7	536	514	1050	51.0	136.3	124
	2° 30'	343	45	5.98	29.6	3.62	18.0	2.36	11.6	539	496	1035	52.0	138.5	110
	4°	548	95	5.98	29.6	3.67	18.2	2.31	11.4	539	496	1035	52.0	138.5	90
T 93 13.7 kgm	5° 30'			5.98	29.6	3.75	18.6	2.23	11.0	539	496	1035	51.7	138.8	135
	7°			6.12	30.3	3.77	18.7	2.35	11.6	539	496	1035	51.5	138.5	134
	Injected blood			5.86		3.25		2.61					30.4	81.5	

# BLOOD CHANGES WITH COMBINED CONDITIONS

TABLE IV (continued)

Experiment number and weight	Time from beginning	Fluid given	Total histamine	Total protein		Albumin		Globulin		Blood volume			Hema-tocrit	Hema-globin	Mean blood pressure
				Serum	For total serum volume	Serum	For total serum volume	Serum	For total serum volume	Red blood cells	Plasma	Whole			
		cc	mgm	per cent	grams	per cent	grams	per cent	grams	cc.	cc	cc.	per cent	per cent	mm Hg
T 94 128 kgm	Control	0	0	7.13	48.1	3.92	26.5	3.21	21.6	[570]*	[675]*	[1245]*	45.8	100.0	112
	1°	128	25	6.81	34.9	3.98	20.4	2.83	14.5	567	512	1079	52.4	115.4	90
	2° 30'	320	60	6.93	34.0	3.87	19.0	3.06	15.0	572	491	1063	53.8	117.1	97
	4°	512	100	6.62	33.1	3.82	19.1	2.80	14.1	563	500	1063	53.0	117.1	73
	5° 30'			7.20	34.1	4.00	19.0	3.20	15.1	564	473	1037	54.4	120.0	116
	7°			6.97	33.1	3.78	18.0	3.19	15.1	562	475	1037	54.2	120.0	103
	Injected blood			5.94		3.53		2.41					30.0	66.1	

\* Determined directly by the dye method

## Protocols

Morphine as anesthetic in all experiments

- T 91 Weight of extremity into which fluid was injected was 2085 grams Weight of opposite extremity 1680 grams  
 Difference 405 grams Total fluid injected was 532 cc Total amount of urine was 68 cc
- T 93 Weight of extremity into which fluid was injected was 2100 grams Weight of opposite extremity 1670 grams  
 Difference 430 grams Total fluid injected was 548 cc Total amount of fluid absorbed was approximately 118 cc  
 Total urine 63 cc with a total protein equivalent of 4.2 grams Stomach contained 370 cc of fluid at completion of experiment with a total protein equivalent of 1.6 grams
- T 94 Weight of extremity into which fluid was injected was 2020 grams Weight of opposite extremity 1620 grams  
 Difference 400 grams Total fluid injected was 512 cc Total amount of fluid absorbed was approximately 112 cc  
 Total urine 26 cc Stomach contained 335 cc of darkly bile-stained fluid with a total protein equivalent of 9.9 grams

was injected was absorbed and the greater part of this could be accounted for by the urine that was passed

The findings in the experiments in which the intestines were traumatized and those in which histamine was injected were quite similar. In each there was an increase in the concentration of the red blood cells, an increase in the percentage of hemoglobin, a decrease in the volume of plasma, very little alteration in the percentage of the protein constituents in the blood serum and a marked decrease in the absolute amounts of each. The proportion of the fluid that was absorbed was approximately the same in the two types of experiments. The percentage of fluid that was absorbed was less with these animals than with the normal ones. Similar experiments (1, 3) previously performed in which fluids were introduced intravenously instead of subcutaneously showed a decrease in the concentration of the protein constituents in the blood serum but otherwise essentially the same findings. It seemed to require more trauma to produce a given decline in the blood pressure when fluids were administered subcutaneously than was necessary in similar experiments in which no fluid was introduced.

In the studies on the effects of graded hemorrhages and the subcutaneous injection of normal salt solution, there was a decrease in the concentration of the red blood cells. The decrease was not quite as great as was usually found when the intravenous introduction of salt solution accompanied hemorrhage. The decrease in the plasma volume was not as great as the quantity of plasma removed. The decrease in the percentage of protein in the serum was less than was found when the fluid was given intravenously. If the protein that was removed with the blood is included, there was a definite increase in the absolute amount of plasma protein. This increase varied from five to eight grams in the different experiments.

The fact that the plasma protein increased in the experiments in which salt solution was injected subcutaneously in normal dogs and in dogs that were bled is of interest from a physiological viewpoint. The question arises as to whether the increase can be explained on the basis of osmosis alone or whether it is necessary to include backward filtration as accounting for part of it. This question we fear cannot be answered from our experiments and no attempt will be made to do so. The recent experiments of Field and Drinker (9) give information on this point. They state, "1 The capillaries under normal conditions are not concerned with the absorption of protein from the subcutaneous tissues. 2 After plasmapheresis, with substantial reduction of total blood protein, foreign protein placed in the subcutaneous tissues can be detected serologically in the blood when entrance by lymphatic routes has been blocked."

Speculation as to the comparative values of administering fluids intravenously and subcutaneously is not without interest. The intravenous

route presents a disadvantage in some instances in which there is a marked decline in the blood pressure in that a marked decrease in the percentage of plasma protein results which is not due to an increase in the plasma volume and hence the osmotic pressure in the blood vessels is lowered. This disadvantage apparently does not exist when the decline in pressure results from hemorrhage. The main objection to the subcutaneous introduction of fluids is that the absorption is slow and especially so when the blood pressure is at a low level. This latter method is not as apt to result in a reduction of the percentage of protein in the serum. It would seem in the absence of a favorable response in the blood pressure following the intravenous introduction of a moderate amount of a solution such as normal salt solution that the injection should be discontinued before a marked decline in the concentration of protein is produced. Possibly the subcutaneous injection of fluids would be of some assistance in maintaining the level in pressure until arrangements for a blood transfusion could be made. Certainly the subcutaneous injection of fluids will tend to prevent the drop in pressure following procedures that are frequently associated with a slow decline.

#### SUMMARY

The effects on the composition of the blood of the subcutaneous introduction of normal salt solution into dogs have been determined repeatedly under the following experimental conditions (1) control studies on the injection alone, (2) trauma to the intestines, (3) the graded removal of blood and (4) the subcutaneous injection of histamine. The studies included determinations of the arterial pressure, the percentage of hemoglobin, the concentration of the red blood cells, the blood volume, the percentages of total protein, albumin and globulin in the blood serum and the volume of salt solution absorbed by the circulation.

The following are some of the results that were obtained

- 1 In normal animals in which salt solution was injected under the skin, there was a slight increase in the volume of plasma, practically no alteration in the concentration and an increase in the absolute amounts of the protein constituents

- 2 Trauma to the intestines and the subcutaneous injection of histamine were associated with a decrease in the volume of plasma, no definite change in the concentration of total protein, albumin and globulin and a marked decrease in the absolute amounts of the protein constituents. A smaller amount of the salt solution was absorbed by the circulation in these experiments than in the other ones

- 3 The graded removal of blood was associated with a decrease in the concentration of the red blood cells, a slight diminution in the percentages of total protein, albumin and globulin in the blood serum, and an increase in the absolute amounts of the protein constituents if the amount of blood removed is included

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# STUDIES IN THE METABOLISM OF SODIUM $\gamma$ -LACTATE I RESPONSE OF NORMAL HUMAN SUBJECTS TO THE INTRAVENOUS INJECTION OF SODIUM $\gamma$ -LACTATE

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In recently published studies, Hartmann and Darrow (1, 2, 3) emphasized the fact that if sodium bicarbonate were properly administered along with other indicated therapeutic measures, severe acidosis could be much more effectively treated. There were recognized, however, a number of objections to the administration of sodium bicarbonate. The most serious was that, if given intravenously in amounts large enough to insure effectiveness, it tended to produce too rapid a change in the reaction of the body fluids, and often resulted in an almost immediate shift from uncompensated acidosis to uncompensated alkalosis, even though the sodium bicarbonate content of the body fluids was not made abnormally high. i.e., the ratio  $\frac{\text{BHCO}_3}{\text{H}_2\text{CO}_3}$  increased because the numerator

was added to more rapidly than the denominator could be increased by the production of carbon dioxide in the tissues and its accumulation in the body fluids through reduced pulmonary ventilation. This danger was usually avoided by giving the alkali in fractional dosages and checking the effect by chemical examination of the blood. The latter constituted another objection, particularly in the case of the young infant. Other disadvantages lay in the fact that if sodium bicarbonate were to be injected subcutaneously or intraperitoneally, it had first to be sterilized by Berkefeld filtration, and then rendered less irritatingly alkaline by bubbling carbon dioxide through it<sup>1</sup>. Unless sealed, such a mixture would of course become too alkaline again as a result of loss of carbon dioxide. Another disadvantage lay also in the fact that the injection of sodium bicarbonate alone tended, at least theoretically, to disturb the ionic balance between sodium, potassium, calcium and magnesium.

In order to overcome those objections the mixture of sodium lactate and hypotonic Ringer's solution was devised (5). The conversion of

<sup>1</sup> Cunningham and Darrow (4) have recently overcome this objection by partly neutralizing sodium bicarbonate previously sterilized in the dry state with hydrochloric acid.

sodium lactate into sodium bicarbonate, it was felt, would be sufficiently slow to permit maintenance of the proper  $\frac{\text{BHCO}_3}{\text{H}_2\text{CO}_3}$  ratio, which would lessen the danger of alkalosis and possibly even permit quantitative restoration of diminished body fluid sodium bicarbonate by a single dose. Sodium lactate, alone or combined with Ringer's solution, is non-irritating, stable and can be sterilized readily by boiling.

The clinical results with this mixture of salts have been more than gratifying, particularly in connection with its use in the treatment of infants with acidosis resulting from diarrhea, dehydration and oliguria. Nevertheless, we have felt it wise to study more thoroughly the response to sodium lactate under various abnormal conditions, and to try to determine the limits of its effectiveness and safety. It seemed quite possible, for instance, that the complete metabolism of sodium lactate with conversion into sodium bicarbonate might be seriously impaired when liver damage or anhydremia and anoxemia were present. It also seemed more than likely that, when given in excess to subjects with diminished body fluid electrolytes or renal insufficiency, sodium bicarbonate might remain in excess in the body fluids, leading to dangerous alkalosis.

To determine the fate of intravenously injected sodium *r*-lactate in normal or essentially normal individuals, children who either had completely recovered from an acute illness or who were suffering from some minor disease were selected from the wards of the St. Louis Children's Hospital. In all instances these subjects were apparently normal in respect to the circulation of the blood and hepatic and renal functions. The procedure was as follows:

In the fasting state in the morning, a control sample of venous blood was secured for determination of carbon dioxide content, glucose, lactic acid and inorganic phosphate. The chemical methods were the same as those used previously (6). Faintly alkaline sodium *r*-lactate<sup>2</sup> was then injected intravenously, in a dose of approximately 4 to 7 cc. of molar solution per kilogram of body weight. To avoid the effects of a too hypertonic solution, the molar preparation was usually diluted with 1 to 2 volumes of distilled water or hypotonic Ringer's solution before the injection. The latter usually required about 30 minutes. Blood samples were again secured for chemical determinations, usually 15, 30, 60, 120 and

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<sup>2</sup> Molar sodium *r*-lactate was made by neutralizing concentrated (approximately 10 molar) lactic acid with approximately 50 per cent sodium hydroxide, with phenol red as the indicator. Because of the anhydride present in the concentrated preparation, the latter was first diluted to about 8 volumes with distilled water and kept boiling slowly, alkali being added to slight excess until all the anhydride was hydrolyzed and neutralized, which usually required from 30 to 45 minutes. A final dilution of 1:10 was then made.

240 minutes after the end of the injection. During this period urine specimens were collected and kept under oil until their carbon dioxide contents could be determined.

## RESULTS

**Blood lactate** Although no check was made by determining the blood lactic acid immediately after injection, the quantity of sodium  $\gamma$  lactate was such as to raise the blood lactic acid approximately 300 to 500 mgm per 100 cc. The rapid fall which occurs during the first 15 minutes after injection is sufficient to reduce such a concentration to approximately 30 to 65 mgm per 100 cc (Table 1 and Chart 1). In other

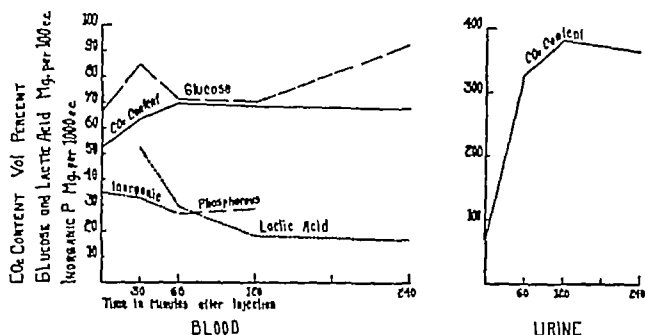


CHART 1 THE AVERAGE RESPONSE OF NORMAL SUBJECTS TO THE INTRA VENOUS ADMINISTRATION OF 6.75 CC OF MOLAR SODIUM  $\gamma$  LACTATE PER KILOGRAM OF BODY WEIGHT

(Average of Cases G 336, G 1982, F 2131 and H 925)

words, about 90 per cent of the injected lactate has left the blood stream during the first quarter of an hour. The disappearance of lactate from the blood then continues at a somewhat slower rate. After one half hour its concentration is usually slightly greater than before the injection, while after one hour it is practically normal. At the end of two hours a subnormal level is often reached.

The initial rapid fall is probably the result of several factors (1) diffusion into the body fluids, (2) oxidation and conversion into glycogen, and (3) excretion into the urine. The latter is probably the least important as but relatively little lactic acid appears in the urine. Quantitative study of this phase is in progress.<sup>1</sup> The later more gradual fall seems due

<sup>1</sup> The amount excreted seems to vary with the rate of injection and the urinary volume. The greatest amount excreted, which we have observed to date, is about 18 per cent.

TABLE 1

*Response of normal human subjects to the intravenous injection of sodium r-lactate*

Case number	Age	Weight	Molar sodium <i>r</i> lactate		Blood						Urine		
			Dose and diluent *	Per kgm of body weight	Minutes after injection	Type of blood sample †	CO <sub>2</sub> content volumes per cent	Lactic acid mgm per 100 cc	Glucose		Inorganic phosphorus mgm per 100 cc	Minutes after injection ‡	CO <sub>2</sub> content volumes per cent
									True	Apparent			
G-2078	5	18.5	130	cc 7 0	0 15 30 60 120	WB WB WB WB WB	52 1 63 5 66 6 65 3 64 5	17 8 63 0 53 6 18 9 12 6	70 0 73 0 95 0 64 0 64 0	73 0 87 0 106 0 73 0 73 0			
G-2155	12	33.9	140	cc 4 1	0 15 30 60 120	WB WB WB WB WB	54 7 59 1 60 9 64 5 59 1	16 4 38 4 32 7 21 4 16 4	56 0 86 0 86 0 83 0 58 0	67 0 99 0 99 0 113 0 87 0		0 30 60 120	40 0 127 0 273 0 35 3
G-2114	9	27.3	110	4 0	0 15 30 60 120	WB WB WB WB WB	53 7 57 6 57 0 58 6 57 1	24 5 41 6 23 0 16 4 11 5	107 0 122 0 110 0 70 0 62 0	112 0 125 0 112 0 73 0 64 0		0	3 1
G-336	14	42.6	245 + 200 R	5 8	0 0 30 30 60 60 120 120 240 240	P WB P WB P WB P WB P WB	56 4 51 1 64 0 56 4 75 9 66 6 70 0 64 9 65 5 62 4	27 7 63 6 30 3 30 3 20 1 16 4	56 4 76 0 76 0 76 0 65 0 76 0	70 0 99 0 86 0 80 0 80 0 86 0	4 0 3 8 2 8 3 3 3 5	0 60 120 240	3 1 350 0 584 0 360 0

TABLE 1 (continued)

Case number	Age	Weight	Molar sodium r lactate		Minutes after infection	Type of blood sample †	CO <sub>2</sub> content	Blood			Urine	
			Dose and diluent *	Per kgm. of body weight				Lactic acid	Glucose		Inorganic phosphorus	Minutes after infection ‡
	years	kgm.	cc.	cc.			volumes per cent	mgm. per 100 cc.	True	Apparent	mgm. per 100 cc.	volumes per cent
G-1982	6	15.0	70 + 210 W	4.7	0	S	63.0	21.4		99.0		
					15	S	77.5	65.6		90.0		
					30	S	93.4	46.7		90.0		
					60	S	80.0	41.7		82.0		
					120	S	60.0	31.5		90.0		
F 2131	8	22.7	170 + 200 R	7.5	0	WB	40.8	22.6	76.0	83.0		0
					30	WB	63.6	32.4	93.0	95.0		
					60	WB	61.0	18.0	79.0	90.0		60
					120	WB	59.9	15.1	85.0	90.0		120
					240	WB	59.4	17.6	90.0	103.0		240
H 925	12	35.5	240 + 250 R	6.8	0	P	57.6		63.0	58.0?	2.9	0
					30	P	70.0	26.5				
					30	WB		60.0	76.0	89.0	2.8	30
					60	P	86.0					
					60	WB		51.0	67.0	80.0	2.5	105
					120	P	84.3					
					120	WB		25.5	114.0	124.0	2.5	240
					240	P	82.8					

\* Diluent = Ringer's solution (R) or distilled water (W)

† S = Serum

WB = Oxalated whole blood

P = Oxalated plasma

‡ Indicates the end of the interval during which urine was collected.

chiefly to the metabolism of the lactate radicle, as indicated by the behavior of glucose and bicarbonate

*Blood glucose* During the first half hour after the intravenous injection of sodium *r*-lactate the glucose concentration rises, the average greatest increase of "true" glucose during this time being 20 mgm per 100 cc (Case G-1982, Table 1, is excluded as no "true" glucose values were determined) During the second half hour its concentration either remains stationary or begins to fall During the second hour a fall usually occurs and a level approximating the initial concentration is usually reached Later there is a distinct tendency to rise

The "true" glucose values were estimated by precipitating the blood proteins with the zinc sulphate reagent of Somogyi (7) and determining the reducing value of the filtrate with the Shaffer-Hartmann reagent (8) in its original form or as modified by Scharles and West (9) The "apparent" glucose values were determined by the original Shaffer-Hartmann procedure (8) Lactate itself in the concentration present during these studies, has no reducing effect on either reagent

In general the "true" and "apparent" glucose values fluctuate in a parallel fashion

*Blood inorganic phosphate* Inorganic phosphate was followed in only two instances (Case G-336 and Case H-925, Table 1) A slight fall was noted during the first half hour, which became more rapid during the second half hour, after which in Case G-336 there was a continued rise In neither instance had the original concentration been reached four hours after injection

*Blood carbon dioxide content* The carbon dioxide content of whole oxalated blood rises in a fashion similar to the increase of "true" glucose The peak is reached usually at the end of the second half hour, although in two instances, Cases G-2078 and G-1982 (Table 1) the highest value was noted at the end of the first half hour During the second hour and the subsequent two hours the concentration falls steadily, due largely to excretion of bicarbonate into the urine The concentration at the end of four hours is not always as low as the initial concentration, but is usually within the upper range of normal The serum and plasma carbon dioxide content values (Case G-336, Table 1) almost parallel the whole blood values, the curves diverging a little as the values increase and converging as they later decrease—in all probability a Donnan equilibrium effect, secondary to increasing and decreasing alkalinity

*Carbon dioxide content of the urine* The carbon dioxide content of the urine begins to rise at almost the same time that the carbon dioxide content of the blood rises The greatest concentration usually appears after about one hour The highest value was observed in Case G-336, Table 1 During the second hour the carbon dioxide content was 584 volumes per cent, representing a concentration of sodium bicarbonate of approximately 21.5 grams per liter

## DISCUSSION

From the above results it would seem that under normal conditions the intravenous injection of molar sodium  $\gamma$  lactate solution in a dose of from 4 to 7 cc. per kilogram of body weight is followed by practically complete utilization of the lactic acid during the period of two hours following the injection. The maximal amount, if injected uniformly over a period of 30 minutes, would correspond to the injection of  $d$ - and  $l$ -lactic acid at a rate of 0.63 gram of each per kilogram per hour. If all of the lactate is metabolized, as is indicated by the small amount excreted into the urine and by the extent of increase of bicarbonate in the blood, one would expect from the work of Meyerhof and Lohmann (10) and Cori and Cori (11) and their associates that about 80 per cent of the  $d$  lactate (corresponding to 0.5 gram of lactic acid per kilogram per hour) would be converted into liver glycogen, while all of the  $l$ -lactate together with 20 per cent of the  $d$  lactate (corresponding to 0.76 gram of lactic acid per kilogram per hour) would be oxidized. The heat production for this amount of lactic acid oxidation would correspond to about 3 calories per kilogram per hour, approximately double the basal metabolic rate of normal children.

If we assume that two-thirds of the body weight represents the weight of body water in equilibrium with and including the blood, we can calculate the expected increase in carbon dioxide content quite readily. Thus, if a subject receives 6.7 cc. of molar sodium  $\gamma$  lactate per kilogram of body weight, there should be an increase of 10 cc. of molar sodium bicarbonate per kilogram of body water, equivalent to an increase in carbon dioxide content of approximately 22.4 volumes per cent. In Table 2 the expected

TABLE 2

*Effect of intravenous administration of sodium  $\gamma$  lactate on the CO<sub>2</sub> content of whole blood of normal human subjects*

History number	Age	Body weight	Body water	Molar sodium lactate	CO <sub>2</sub> content			Difference
					Before	After 1 hour	Expected	
	years	kgm.	liters	cc.	volumes per cent	volumes per cent	volumes per cent	volumes per cent
G-2078	5	18.5	12.4	130	48.5	67.6	75.6	- 10.3
G-1982	6	15.0	10.0	70	63.0*	80.0*	78.7	+ 1.3
F 2131	8	22.7	15.2	170	49.8	61.0	74.9	- 13.9
G-2114	9	27.3	18.2	110	53.7	58.6	67.2	- 8.6
G-2155	12	33.9	22.7	140	54.7	64.5	68.5	- 4.0
G-336	14	42.6	28.6	245	51.1	66.6	70.3	- 3.7
"		42.6	28.6	245	56.4*	75.9*	75.6	+ 0.3
H 925	12	35.5	23.7	240	57.6	86.0	80.3	+ 5.7
							Average = - 4.2	

\* Determinations on serum



increase in carbon dioxide content of the blood is compared with that actually observed after one hour. It should be noted that in all instances but two the actually observed carbon dioxide content was less than that predicted, and that the average discrepancy was 4.2 volumes per cent. The chief reason for this discrepancy undoubtedly is the fact that considerable base, chiefly as bicarbonate, is excreted into the urine during the first hour. In Case G-336 during the initial hour the carbon dioxide content of the urine rose from 3.1 to 35.0 volumes per cent. The quantity of urine secreted during the interval was 100 cc. accounting for the excretion of approximately 15.5 cc. of molar sodium bicarbonate. As the subject had approximately 28.6 kilograms of body water, the loss of 15.5 cc. of molar sodium bicarbonate would cause a reduction in the carbon dioxide content of the entire body water of 1.21 volumes per cent. The difference originally to be accounted for in this instance was 3.7 volumes per cent. Undoubtedly, additional base (sodium) was excreted with other anions, such as chloride and sulphate.

The most alkaline urine observed in this study was noted during the second hour in this same subject. During this period, 150 cc. of urine with a carbon dioxide content of 58.4 volumes per cent were secreted, representing a concentration of sodium bicarbonate of approximately 21.5 grams per liter. In no instance was any clinical evidence of alkalosis, such as tetany, noted despite the fact that in one instance (Case G-1982 Table 1) a serum carbon dioxide content of 93.4 volumes per cent resulted. In all probability the respiratory response was sufficient to prevent any significant shift in pH.

### SUMMARY AND CONCLUSIONS

In seven essentially normal children the effects of the intravenous injection of from 4 to 7 cc. of molar sodium *r*-lactate per kilogram of body weight were studied, with the following results:

- 1 The racemic mixture is practically completely metabolized in from one to two hours.

- 2 The conversion of lactate into glucose is apparent from the uniform rise of the latter in the blood.

- 3 The liberation of the sodium ion is practically quantitative, the base appearing in the body fluids chiefly as sodium bicarbonate.

- 4 Excretion of excess base into the urine takes place promptly, and alkalosis is usually of short duration.

- 5 During the duration of alkalosis no signs of tetany were noted.

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# STUDIES IN THE METABOLISM OF SODIUM *r*-LACTATE II RESPONSE OF HUMAN SUBJECTS WITH ACIDOSIS TO THE INTRAVENOUS INJECTION OF SODIUM *r* LACTATE

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In the first paper of this series it has been demonstrated that sodium *r*-lactate is completely metabolized within a period of from one to two hours by normal individuals when given in a dose of 7 cc of molar solution per kilogram over a period of one-half hour. The utilization of the racemic mixture of lactate probably involves the oxidation of a fraction of the lactate radicle and the conversion into glycogen of the remainder, as well as the liberation of the sodium ion, which then is excessive in the body fluids and is excreted into the urine.

In the present study the effect of intravenous sodium lactate upon the carbon dioxide content of the blood in patients with acidosis was investigated. For the sake of convenience, the cases have been divided into three groups (1) those with acidosis secondary to renal insufficiency, (2) those with acidosis associated with diarrhea, dehydration and oliguria, and (3) those associated with diabetes mellitus.

## *Nephritic acidosis*

The acidosis associated with renal insufficiency is brought about rather simply, being due almost entirely to the accumulation of such acids as phosphoric and sulphuric, and to the loss of fixed base from the body fluids, because of the impairment of renal function (1). Except in the terminal stages the circulation of the blood is practically normal, as is also apparently the ability on the part of the subject to oxidize and store carbohydrate. This type of acidosis, therefore, from theoretical considerations, should be promptly relieved by the administration of sodium lactate in sufficient amounts. In Table 1 are recorded the results to date of treatment of this type of acidosis with sodium *r* lactate. It should be noted that, except in one instance, the expected increase in the carbon dioxide content was fairly close to that actually observed, and in the averages of four instances cited there was a discrepancy of only 1.6 per cent. In the one instance in which this discrepancy was considerably greater ( $-13.4$  volumes per cent) the explanation may be that only one

TABLE 1  
*Effect of intravenous administration of sodium  $\gamma$ -lactate on plasma  $\text{CO}_2$  content*  
*Cases of acidosis associated with chronic nephritis*

History number	Age years	Date	Body weight kgm	Body water liters	Molar sodium lactate cc	Plasma $\text{CO}_2$ content			Difference	Remarks
						Before	After ( ) hours	Expected		
G-198	7	October 24, 1930	17.0	11.4	112	20.8	51.3 (4)	42.8	+ 8.5	Chronic suppurative nephritis associated with urethral obstruction and cystitis
		October 30, 1930	17.2	11.5	110	33.1*	41.1*(1)	54.5	- 13.4	
		February 6, 1931	18.1	12.1	100	30.1*	48.4*(2)	48.9	- 0.5	
0	33	December 1, 1930	60 $\pm$	40.2	240	36.8	49.3(2)	50.2	- 0.9	Chronic glomerular nephritis
								Average	- 1.6	

\* Determinations on whole blood

hour elapsed after the beginning of the injection and the taking of the next blood sample

*Acidosis associated with diarrhea, dehydration and oliguria*

This type of acidosis results from more complex factors than does the nephritic type. As in the latter, there is always loss of fixed base from the body fluids. There is also a loss of acid radicles, and in some instances water loss is so great that despite the loss of minerals from the body fluids the actual chloride and total base concentrations observed may be greater than normal. Associated with the diminished volume and flow of the blood, there is a diminished urinary secretion, and while it lasts the same factors that contribute towards the formation of acidosis seen in chronic renal insufficiency are at work. Occasionally, particularly in the restless infant or the infant with convulsions, lactic acid accumulates in the body fluids. Such an accumulation is looked upon as due essentially to anoxemia, which seems due largely to the reduced blood flow. Aside from these considerations another factor should be mentioned, at least. At autopsy evidence of liver damage is frequently noted in this type of patient. It is quite possible that such liver damage might interfere with the normal metabolism of lactic acid diffusing from the muscles into the blood stream.

In Table 2 are included all the results to date of the treatment of this type of acidosis with carefully measured amounts of the racemic lactate preparation<sup>1</sup>. It should be noted that in all six instances, although an increase in the carbon dioxide content occurred, which brought the patient well out of the danger zone of acidosis, nevertheless, the observed rise fell short of that predicted. The discrepancy varied from 5.5 to 24.9 volumes per cent, the average being 12.1 volumes per cent.

There are probably a number of factors contributing to this discrepancy. Because of the dehydration of the body the administration of a watery solution of sodium lactate will tend to dilute the body fluids, and by dilution to reduce the concentration of the salts (including sodium bicarbonate) present. Then, as has been shown previously (2) acidosis of this type is frequently uncompensated and low pH values may occur. With the partial restoration of sodium bicarbonate to the body fluids there would be of course a restoration of pH which would tend to cause a withdrawal of a certain amount of base bound to bicarbonate for combination with other ions, such as protein. In addition to these factors, the

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<sup>1</sup> The use of the mixture of sodium  $r$  lactate and hypotonic Ringer's solution made by Eli Lilly and Company and designated as Physiological Buffer Salts Solution has been routine in the wards of the St. Louis Children's Hospital for more than two years. Use of this very much larger amount of material in this paper is impossible because of the minimal amount of blood chemistry study which seemed required in the successful treatment of these patients.

TABLE 2

*Effect of intravenous administration of sodium r-lactate on plasma CO<sub>2</sub> content  
Cases of acidosis associated with diarrhea—dehydration—oliguria*

History number	Age	Date	Body weight	Body water	Molar sodium lactate	Plasma CO <sub>2</sub> content			Difference
						Be-fore	After ( ) hours	Ex-pected	
	months		kgm	liters	cc.	volumes per cent	volumes per cent	volumes per cent	volumes per cent
G-2058	16	October 28, 1930	7.2	4.8	90	27.5	44.6(2)	69.5	- 24.9
G-2352	1	November 23, 1930	2.7	1.8	20	26.9	46.3( $\frac{1}{2}$ )	51.8	- 5.5
G-2127	3	December 14, 1930	4.7	3.2	45	23.5	42.5(3)	55.0	- 12.5
G-814	32	August 5, 1930	9.5	6.4	80	29.5	49.8(2)	56.5	- 6.7
H-479	9	February 28, 1931	7.0	4.7	60	23.6	46.5(3)	52.4	- 5.9
H-669	1	March 14, 1931	2.7	1.8	30	23.9	43.8(3)	61.2	- 17.4
						Average = - 12.1			

original cause of the acidosis, the diarrhea entailing the loss of gastrointestinal secretions, continues to be effective, so that while acidosis is being relieved by administration of alkali, some of this restored alkali is being excreted out into the lumen of the bowel.

An interesting point, which has come to light in the treatment of this type of acidosis, concerns the behavior of the lactic acid concentration of the blood of such cases. Even though lactic acid may be abnormally high before the injection of sodium *r*-lactate, normal values are usually seen two or three hours after the injection. The explanation, in all probability, lies in the effect of the increased blood flow on utilization of lactic acid. Occasionally, however, in the presence of marked disturbance of circulation which persists after the administration of fluids, the metabolism of lactate seems delayed. In such cases the administration of oxygen by means of the oxygen tent seems of value in hastening oxidation.

Another point of extreme interest is the fact that the metabolism of sodium lactate may be accomplished right up to the point of death. Thus, Case G-2058 was dying from a hemolytic streptococcus septicemia, mastoiditis and pyuria when the test was begun (for the therapeutic purpose of relieving the acidosis). Although the patient died about four hours after the administration of the sodium lactate, the plasma carbon dioxide content had increased from 27.5 to 44.6 volumes per cent during the first half of this period. Similar phenomena were encountered in Case G-2127, death here also being due to infection and occurring within twelve hours after relief of acidosis, following the lactate administration.

*Diabetic acidosis*

Severe diabetic acidosis is also due to complex causes (3) One factor of importance is, of course, the accumulation of the organic acids which tend to displace the bicarbonate ion from combination with base Other significant factors are, however, the loss of fixed base from the body fluids, which occurs during the period of polyuria and of vomiting, and the later effect of anhydremia on renal function, which is similar to the effect of anhydremia in the dehydrated infant with diarrhea, that is, essentially there is renal insufficiency which permits phosphoric and sulphuric acid to accumulate and which prevents excretion of these anions and others, such as chloride and the ketone acids, free of base or bound to ammonia In addition to these factors there is, of course, the altered carbohydrate metabolism, secondary to insulin insufficiency

In Table 3 are recorded the results to date of the treatment of this type of acidosis with sodium *r* lactate. If we consider first the results obtained when no insulin was administered simultaneously, we note that in Case G 1214 who was not in a very critical condition, the rise of the carbon dioxide content of the blood after three hours was somewhat in excess (8.8 volumes per cent) of that predicted In all probability, therefore, in addition to complete release of base bound to the lactate ion, base was also released from other anions In Case G 2520, however, the observed carbon dioxide content was 16.6 volumes per cent less than expected In this instance the acidosis was more severe and the factors inducing the production of acidosis were apparently operating more intensively In this instance, also, there was clinically more dehydration and less diuresis

TABLE 3

*Effect of intravenous administration of sodium *r* lactate on plasma CO<sub>2</sub> content  
Cases of acidosis associated with diabetes mellitus*

Case number	Age	Body weight	Body water	Molar sodium lactate	Plasma CO <sub>2</sub> content			Difference	Remarks
					Before	After ( ) hours	Expected		
	years	kgm.	liters	cc.	volumes per cent	volumes per cent	volumes per cent	volumes per cent	
G-1214	7	25 ±	16.8	120	34.8	59.6(3)	50.8	+ 8.8	No insulin given
G-2520	12	35.0	23.5	235	25.4*	31.2*(2)	47.8	- 16.6	No insulin given
G-1313	2	10.0	6.7	80	29.7	56.9(3)	56.4	+ 0.5	15 units insulin given
H 1054	12	33.7	22.4	210	15.0	42.0(2)	36.0	+ 6.0	40 units insulin given
G-463	11	45 ±	30.1	630	8.5	34.8(2)	56.0	- 21.2	100 units insulin given
Average = - 4.5									

\* Determinations on whole blood



If we turn next to the two instances in which insulin was given along with sodium *r*-lactate, we find that in one case (G-1313), moderately severe acidosis was completely relieved and the observed rise in the carbon dioxide content was almost identical with that predicted. In another (H-1054), the carbon dioxide content rose from 15 to 42 volumes per cent in two hours, the increase being 6 volumes per cent more than expected. In case G-483, however, we find that a marked discrepancy occurred and a much lower carbon dioxide content was observed than predicted, even though two hours had elapsed after the injection of lactate. In addition to the very marked dehydration and acidosis a number of factors seemed responsible for this discrepancy. An unusually large dose of lactate was given (14 cc molar per kilogram of body weight). Two hours after the injection the blood lactic acid was still 100 mgm per 100 cc. At this time the body temperature had risen to 42.8° C, and the pulse was so rapid that it could not be accurately counted. Two hours later, without regaining consciousness, the patient died. At the time of death, approximately four hours after the administration of the lactate, the blood lactic acid was still elevated, 55 mgm per 100 cc. The carbon dioxide content at this time was 36.8 volumes per cent. In all probability the hyperpyrexia was the result of the specific dynamic action of lactate in stimulating metabolism, and to us it seemed that it was an important factor in causing death, although the patient was desperately ill before treatment was started. Until further studies have been made upon the effect of heat production from lactate administration, we feel that a dose of 7 cc of molar solution per kilogram of body weight should not be exceeded, particularly in the diabetic subject who also receives insulin.

In Table 4 are included data which indicate the effect of the intravenous administration of *sodium bicarbonate* on the plasma carbon dioxide content of patients with different types of acidosis. In general, the same type of agreement and discrepancy between the predicted and the observed carbon dioxide contents were noted.

From the data discussed above it seems that one can predict fairly well the effect of any given dose of sodium *r*-lactate on an individual with acidosis, providing the dose is not so large as to let too marked alkalosis develop, which would complicate matters. In applying this data so that it might be of assistance clinically, we have calculated in the following manner the dose of molecular sodium lactate or bicarbonate necessary in individual cases to restore any observed carbon dioxide content of the body fluids to normal within a period of two to three hours.

- 1 Body water (blood, lymph, spinal fluid, muscle water, etc.) equals 60 to 70 per cent (approximately two-thirds) of body weight
- 2  $\text{BHCO}_3$  of entire body water approximates  $\text{BHCO}_3$  of plasma
- 3 One cc molecular  $\text{NaHCO}_2$  per liter of body water equals 2.24 cc of  $\text{CO}_2$  per 100 cc (2.24 volumes per cent  $\text{CO}_2$ )

TABLE 4  
Effect of intravenous administration of  $\text{NaHCO}_3$  on plasma  $\text{CO}_2$  content

Case	Body weight kgm.	Molar NaHCO <sub>3</sub>	Plasma CO <sub>2</sub> content			Differ ence	Plasma pH		Remarks
			Before	After ( ) hours	Expected		Before	After ( ) hours	
A.W	8.26	95	55.7 vol. per cent	96.5(1) vol. per cent	94.2 vol. per cent	+ 2.3	7.47	7.58(1)	Acute "pyelitis" no renal insufficiency
K.P	6.1	60	15.5	64.5(8)	48.3	+ 16.2	7.12		Marked ketosis glucose also administered
R.D	16.2	119	24.0	54.6(16)	48.5	+ 6.1	7.33	7.45(16)	Marked renal insufficiency Ringer's solution also administered
W.S	4.0	75	12.9	43.2(2)	74.4	- 31.2			Diarrhea, dehydration and oliguria
V.N	27.3	119	15.5	23.7(3)	29.5	- 5.8			Diabetic coma insulin also administered
A.S	30.0	95	10.6	16.1(3)	23.2	- 7.1			Diabetic coma insulin also administered
N.G	27.0	150	15.0	40.3(3)	33.7	+ 6.6	7.10		Diabetic coma insulin, glucose and Ringer's solution also administered
E.S	23.6	300	18.7	22.8(3) 92.5(8)	37.7	- 14.9 + 54.8	7.00 { 7.57(8)	7.15(3) 7.43(8)	Diabetic coma, insulin, glucose and Ringer's solution also administered
	23.6	150	7.4	53.8(8)	28.7	+ 25.1	7.05		

- 4 Therefore, expected increase in volumes per cent CO<sub>2</sub> equals  

$$\frac{2.24 \times \text{cc molecular NaHCO}_3 \text{ or Na lactate}}{\text{liters body water}}$$
- 5 Or, dose of molecular NaHCO<sub>3</sub> or Na lactate to increase CO<sub>2</sub> content of  
 body water to 60 volumes per cent equals  $\frac{60 - \text{CO}_2 \text{ content}}{2.24}$   
 $\times$  liters body water A more workable expression of this equation  
 is, dose of molecular Na lactate equals  $(60 - \text{CO}_2 \text{ content}) \times (0.3$   
 $\times$  body weight in kgm )

We feel that this method of calculating the dose of molar sodium *r*-lactate is perfectly safe as regards the treatment of nephritic acidosis, as observed values will agree closely to those predicted. In the case of acidosis associated with diarrhea, dehydration and oliguria, the observed increase will be less than the predicted, but in all probability, sufficient to bring the patient well out of the danger zone. In the case of diabetic acidosis the other measures of value in the treatment of acidosis, such as administration of insulin, carbohydrate and Ringer's solution, should, of course, be carried out. In this type of acidosis, because of the synergistic effect of insulin, the dose of sodium lactate calculated to raise the carbon dioxide content 15 to 20 volumes per cent should be sufficient and should not result in a dangerous increase in heat production.

### SUMMARY AND CONCLUSIONS

The response of subjects with acidosis to the intravenous injection of sodium *r*-lactate was studied, with the following results

- 1 Sodium *r*-lactate is metabolized in practically a normal fashion in subjects with acidosis
- 2 In the three chief types of acidosis (nephritic, diarrheal and diabetic) the increase in the carbon dioxide content of the blood can be fairly accurately predicted

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# STUDIES IN THE METABOLISM OF SODIUM *r*-LACTATE III RESPONSE OF HUMAN SUBJECTS WITH LIVER DAMAGE, DISTURBED WATER AND MINERAL BALANCE, AND RENAL INSUFFICIENCY TO THE INTRAVENOUS INJECTION OF SODIUM *r*-LACTATE

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Since the complete metabolism of sodium *r*-lactate involves to such a great extent hepatic and renal activity, it should naturally be of interest and importance to study the response of subjects with liver and kidney damage

## *Acute catarrhal jaundice*

Up to the present time we have been able to study four cases of *acute hepatitis* of the type more commonly known as acute catarrhal jaundice. As may be noted from Table 1 and Chart 1, the rate of disappearance of lactate from the blood was considerably delayed in the subjects with jaundice as compared with the normal. Increase in glucose in the blood was less rapid and less marked. A slight delay in the rise of the carbon dioxide content of the blood was also noted, the peak coming at the end of the second hour in two instances, and at the end of the fourth hour in one. In order to compare these data graphically with the normal, the results of Cases H-1130, F-2131 and H-925 were averaged because of their more complete data and because the dose of molar sodium *r*-lactate was approximately the same in each instance, averaging almost exactly 7 cc per kilogram of body weight. For the graphic presentation of the normal subjects Cases G-336, G-1982, F-2131 and H-925 were selected for similar reasons. The average dose of molar sodium *r*-lactate in this group was 6.75 cc per kilogram of body weight.

## *Hemolytic jaundice*

The response of Case G-2473 (Table 2) resembled that of the patients with acute hepatitis in the delayed blood carbon dioxide content peak. This patient had just recovered from a "crisis" of abdominal pain, fever and vomiting. The liver was enlarged, its edge reaching the level of the umbilicus. In all probability some impairment of liver function was present.

TABLE 1  
*Acute hepatitis*

Case number and date	Age	Weight	Molar sodium $\gamma$ lactate		Mln utes after in jection	Type of blood sample†	Blood						Urine			Remarks
			Dose and diluent*	Per kgm body weight												
G-2144 October 21, 1930	9	24.6	110 + 180 W	4.5												Onset of jaun- dice October 18, 1930
					0	S	mgm per 100 cc	Lactic acid	mgm per 100 cc	True	Apparent	Inor- ganic phos- phorus	Time of urine collec- tion†	CO <sub>2</sub> con- tent	Vol- ume	
					15	S	71.6	21.4	74.0	126.0	126.0	mgm per 100 cc		vol- umes per cent	cc	
					30	S	70.0	42.8	71.0	151.0	151.0					
					60	S	72.7	31.5	71.0	172.0	172.0					
					120	S	73.7	21.3	86.0	126.0	126.0					
October 24, 1930					180	S	73.2	16.3	80.0	99.0	93.0					Jaundice still present, di- minishing rapidly
						WB	53.7	17.6	74.0	100.0	100.0					
					15	WB	59.4	29.7	71.0	87.0	87.0					
					30	WB	59.9	23.9	71.0	87.0	87.0					
November 17, 1930					60	WB	62.8	15.1	86.0	87.0	87.0					Completely re- covered clin- ically
					120	WB	58.1	12.6	80.0	87.0	87.0					
						WB	53.7	24.5	107.0	112.0	112.0		0	4.1		
					15	WB	57.6	41.6	122.0	125.0	125.0		30	45.4		
H-1130 May 16, 1931	3	16.7	115 + 120 R	6.9	30	WB	57.0	23.0	110.0	112.0	112.0		60	359.0		Onset of jaun- dice May 14, 1931
					60	WB	58.6	16.4	70.0	73.0	73.0		120	309.0		
					120	WB	57.1	11.5	62.0	64.0	64.0					
						WB	59.2	20.2	52.0	58.0	58.0	4.8	0	8.2		
					50	WB	68.2	76.7	126.0	133.0	133.0	4.5	60	172.0	120	
					120	WB	75.2	25.2	111.0	119.0	119.0	3.9	150	250.0	100	
					240	WB	67.8						240	288.0	70	

TABLE 1 (continued)

TABLE 1 (continued)

Case number and date	Age	Weight	Molar sodium $\gamma$ -lactate		Mn-utes after in jection	Type of blood sample†	Blood					Urine			Remarks
			Dose and diluent*	Per kgm body weight			CO <sub>2</sub> content	Lactic acid	Glucose		Inor-ganic phos-phorus	Time of urine collec-tion ‡	CO <sub>2</sub> con-tent	Vol-ume	
June 25, 1931		kgm	cc	cc			vol-umes per cent	mgm per 100 cc.	mgm per 100 cc	mgm per 100 cc	mgm per 100 cc		vol-umes per cent	cc	Completely re-covered clin-ically
			240	6 8	0	P	57 6	25 2	63 0	58 0	2 9	0	22 1		
			+		0	WB	70 0	58 2	76 0	89 0	2 8	30	99 6	430	
			250 R		30	WB	86 0	46 6	67 0	80 0	2 5				
					60	WB	84 3	18 7	114 0	124 0	2 5	105	311 0	240	
					120	P						240	498 0	210	
					240	P									
					240	WB									

\* Diluent = Ringer's solution (R) or distilled water (W)

† S = Serum

WB = Ovalated whole blood

P = Ovalated plasma

‡ Indicates the end of the interval (in minutes) during which urine was collected

TABLE 2  
Miscellaneous cases

Case number	Age	Weight	Molar sodium lactate		Whole blood					Urine		Remarks
			Dosage and diluent*	Per kgm. of body weight	Min. ures after injection	CO <sub>2</sub> content	Lactic acid	Glucose		Time of urine collection	CO <sub>2</sub> content	
								True	Apparent			
	years	kgs	cc.	cc.		vol. mms per cent	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	minutes	vol. mms per cent	
G-2473	5	18.2	130	7.2	0	52.7	11.0	73.0	99.0	0	11.0	Patient with hemolytic icterus just recovering from 'crisis' of abdominal pain, vomiting and fever. Liver definitely enlarged
					30	61.6	47.8	113.0	125.0	30	65.0	
					60	60.7	20.1	103.0	132.0	60	310.0	
					120	64.3	17.5	83.0	93.0	120	350.0	
					240	56.0	21.5					
G-2099	15	32.7	160 + 275 R	4.9	0	48.5	27.1	60.0	67.0		Diagnosis Unilateral tuberculosis of kidney	
					15	48.5	37.8	99.0	100.0			
					30	66.5	31.5	105.0	113.0			
					60	67.6	15.8	102.0	113.0			
					120	63.7	12.6	90.0	93.0			

\* Diluent = Ringer's solution (R)

† Indicates the end of the interval during which urine was collected



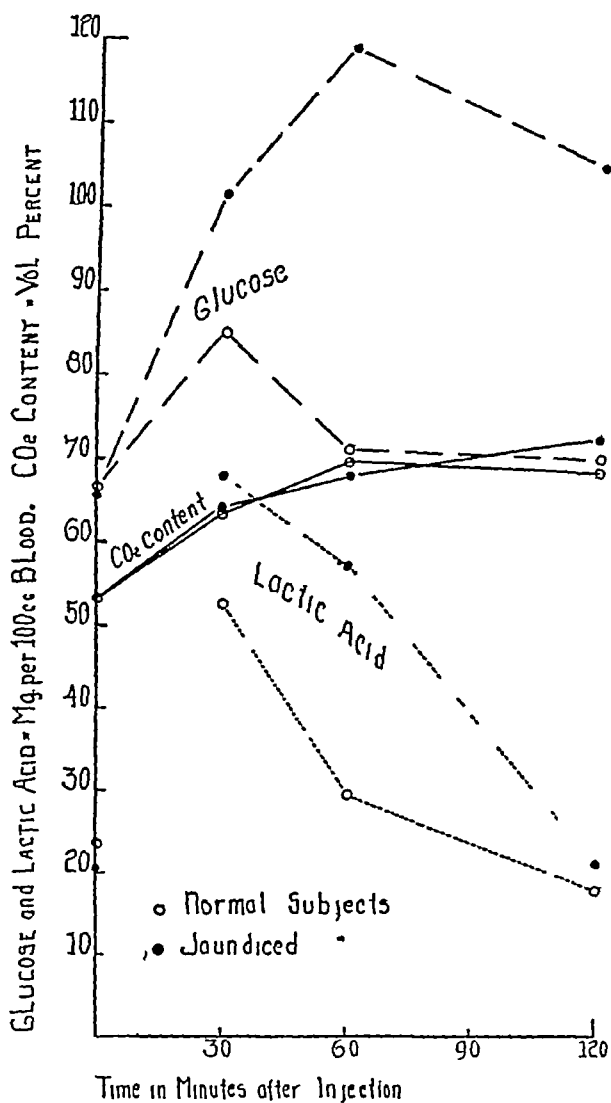


CHART 1 COMPARISON OF RESPONSE OF NORMAL SUBJECTS AND SUBJECTS WITH ACUTE HEPATITIS TO THE INTRAVENOUS INJECTION OF SODIUM *r*-LACTATE

*Anhydremia and hypochloridemia secondary to acute pyelonephritis*

In Case H-695 (L.H.) we were able to study a rather severe urinary tract infection of the acute pyelonephritic variety with a secondary disturbance of water and mineral balance. At the beginning of the study on March 18, 1931, the patient was dehydrated from previous vomiting and fever and showed the effects of loss of gastric juice in the diminished serum chloride content (491 mgm per 100 cc). The nonprotein nitrogen was markedly elevated (160 mgm per 100 cc), and the standard urea clearance (Van Slyke) was 64 per cent of the normal. The carbon dioxide content was at the upper limits of normal (63 volumes per cent). The urine was entirely free from chloride, bicarbonate and lactic acid. We predicted in this case that the injection of the usual amount of sodium *r*-lactate would be followed by a persistent alkalosis with continued acid urine, because of the initially low electrolyte content of the blood (due to loss of chloride) and that later by giving chloride the bicarbonate could be made to enter the urine and alkalize the latter.

As may be noted from Table 3, the first half of our prediction was realized, the carbon dioxide content rose and was still 100 volumes per cent 16 hours after the administration of the lactate. The urine in the meantime remained acid, the highest carbon dioxide content being but 13 volumes per cent.

The following day, after the intravenous administration of 900 cc. of Ringer's solution, the plasma carbon dioxide content began to fall, as expected. In its excretion into the urine, however, base must have been claimed by acids other than bicarbonate, as the bicarbonate content of the urine remained practically at zero. It does not seem improbable, since nonprotein nitrogen was falling rapidly in concentration in the blood, that acid radicals were also being excreted, claiming the base released by bicarbonate.

*Renal insufficiency secondary to chronic pyelonephritis*

In Case G-198 (Table 4) we had the opportunity to study the response of a patient with marked renal insufficiency secondary to long standing cystitis and obstruction of the bladder neck. As discussed previously, it is easy to relieve the acidosis of this type of case by administering properly a calculated dose of sodium *r*-lactate. After this was accomplished on February 6, 1931, we repeated the administration of the lactate. Theoretically the initial carbon dioxide content of 43.1 volumes per cent should have been raised to 71.1 volumes per cent. Actually, this exact concentration was reached after one hour and maintained during the second hour. After four hours, however, the carbon dioxide content had diminished to 61.0 volumes per cent. This fall was not attended by any increased alkalinity of the urine. It is interesting to note that a somewhat similar late fall in the carbon dioxide content of the blood occurred

TABLE 3  
*Acute pyelonephritis with anhydremia and hypochloridemia*  
(Case H-695 Female, age 8 years, weight 23 kgm.)

Date and time relative to injection	Blood							Urine				
	Type of blood sample*	CO <sub>2</sub> content	NaCl	Lactic acid	Inorganic phosphorus	Protein	Calcium	Non-protein nitrogen	Time\$ relative to injection	CO <sub>2</sub> content	NaCl	Lactic acid
minutes		sol- times per cent	mgm per 100 cc	mgm per 100 cc	mgm per 100 cc	grams per 100 cc.	mgm per 100 cc	mgm. per 100 cc	minutes	sol- times per cent	mgm per 100 cc	mgm per 100 cc
March 18, 1931									March 18, 1931			
Before 270'	S	63 0	491		6 5	7 12	12 4	160 0	Before	1 0	0 0	0 0
Just before injection I †	P	53 7		53 5		6 09						
After 30'	P	75 6		78 4				150 0	After 120'	13 0	0 0	92 3
After 70'	P	86 7	421									
After 70'	WB	76 0		41 6	5 0			145 0				
After 135'	S	90 4		45 3	5 4			140 0				
March 19, 1931									March 19, 1931			
Injection II †									Before	4 7	0 0	18 0
Just after	S	100 3	450	31 5								
After 60'	S	91 2	533	35 2				123 0	After 60'	8 8	170	
After 120'	S	88 8	567	32 8					After 120'	5 2	350	
After 240'	S	79 8	527						After 240'	7 8	350	
March 20, 1931												
Early A M	S	90 0	538					100 0				
Noon	S	84 0	515					68 0				

\* S = Serum

WB = Oxalated whole blood

P = Oxalated plasma

† 161 cc molar sodium  $\gamma$ -lactate plus 800 cc distilled H<sub>2</sub>O given intravenously

‡ NaCl 15.0 grams, KCl 0.5 gram, CaCl<sub>2</sub> 0.25 gram in 900 cc distilled water given intravenously

§ Indicates the end of the interval (in minutes) during which urine was collected

TABLE 4  
*Chronic pyelonephritis with renal insufficiency*  
*(Case G-198 Male, age 7 years, weight 16.8 kgm.)*

Date	Molar sodium lactate		Blood						Urine		Remarks
	Dose and diluent*	Per kgm. of body weight	Min- utes after in- jection	Type of blood sample†	CO <sub>2</sub> con- tent	Lactic acid	Glucose		Time of urine collec- tion	CO <sub>2</sub> con- tent	
							True	Apparent			
October 30, 1930	cc.	cc.			vol times per cent	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.		vol times per cent	Whole blood N P N = 87 mgm. per 100 cc.  P S P output = 1 per cent in 2 hours. Maximum urinary concentration = 1 010
	110	6.5	0	WB	33.1	18.9	127	151	0	13.1	
			15	WB	35.2	41.1	158	185	15	11.0	
			15	P	39.3						
			30	WB	40.8	30.9	158	185	30	10.2	
			30	P	46.1						
February 6, 1931			60	WB	41.1	22.7	97	118	60	13.1	Maximum urea clearance (Van Slyke) = 10 per cent
			120	WB	39.8	12.6	62	87	120	12.9	
		6.0	0	WB	30.1	27.5	49	70	0	15.3	
	100 + 100 R		30	WB	41.9	56.7	90	102	45	15.3	
			60	WB	48.4	39.7	76	96			
			120	WB	48.4	27.0	65	83	120	23.0	
			240	WB	40.4	16.0	60		300	38.9	

TABLE 4 (continued)

Date	Molar sodium $\gamma$ -lactate		Blood						Urine		Remarks
			Min-utes of body in-jection	Type of blood sample†	CO <sub>2</sub> con- tent	Lactic acid	Glucose		Time of urine collec- tion†	CO <sub>2</sub> con- tent	
	Dose and diluent*	Per kgm of body weight					True	Apparent			
		cc.	cc.			sol- umes per per cent cc.	mgm per 100 cc	mgm per 100 cc	mgm per 100 cc		
February 7, 1931	150	9 0	0	WB	43 1	12 6		89	0	46 0	Whole blood N P N = 87 mgm per 100 cc
	+		30	WB	64 3	80 7		121			
	100 R		60	WB	71 1	41 0		128	60	36 0	
			120	WB	71 6	30 5		121			
			240	WB	61 0	19 0			180	36 5	

\* Diluent = Ringer's solution (R)

† WB = Oxalated whole blood

P = Oxalated plasma

‡ Indicates the end of interval during which urine was collected

in the two previous instances of lactate administration in this case, even though the carbon dioxide content had not been brought into the zone of alkali excess. Complete mineral balance experiments will have to be done before we can be certain as to the mechanism by which such excess base disappears from the blood of such subjects.

#### SUMMARY AND CONCLUSIONS

The response to the intravenous injection of sodium  $\tau$  lactate of patients with acute hepatitis, acute and chronic pyelonephritis and miscellaneous diseases was studied, with the following results:

- 1 In the four cases of acute catarrhal jaundice studied there was a slight, but definite *delay* in the complete metabolism of the lactate.
- 2 In the case of anhydremia and hypochloridemia secondary to acute pyelonephritis because of initial low electrolyte concentration of the body fluids the base released from lactate was held as bicarbonate, causing persistent alkalosis, which was then relieved by administration of chloride.
- 3 In a single case of marked renal insufficiency secondary to chronic pyelonephritis, alkalosis was of no greater duration than in the normal subject.



## A CELLULOID CAPSULE FOR MEASURING VENOUS PRESSURES

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(Received for publication November 3 1931)

The methods of determining human venous pressure directly by venous puncture and indirectly in various ways have been reviewed by Eyster, 1926, 1929. Among the indirect methods that of applying a capsule to a superficial vein has been most used. The air within the capsule is raised to a pressure sufficient to collapse the vein, a pressure therefore estimated approximately equal to the blood pressure within the vein. The capsules used have been of two kinds, (1) a capsule held by hand against the skin with the help of a glycerine coating, with the vein seen either through a transparent membrane (Carrier and Rehberg, 1923) or through a hole in a thin rubber membrane (Eyster, 1929), (2) a glass capsule with no membrane but attached to the skin around its periphery by collodion (Hooker, 1916). Though these capsules have been very useful, difficulties have been recognized in making accurate determinations. At best it is not easy to be sure of the exact end point and any membrane through which the vein must be observed lessens visibility. In an attempt to measure the high venous pressures in the feet of standing subjects it was found impossible to hold either type of membrane capsule against the skin with sufficient steadiness to secure trustworthy figures. An attached glass capsule, however, is too rigid to fit veins varying in size and arrangement. The skin around and inside such a capsule, also, is deformed conspicuously if a high pressure must be used.

The description of a new capsule and a counterweighting clamp which obviate these difficulties is the purpose of this paper. The essential features of construction and use are these. The capsule is made of celluloid which permits the cutting of notches to fit any vein. Its construction is so simple that a dozen may be made in an hour. A considerable length of vein is under observation. By a clamp for counterweighting there is freedom from the skin deformation incident to any attached capsule at high pressures. Comparable serial readings are possible through any length of time.

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<sup>1</sup> Sarah Berliner Fellow of the American Association of University Women, 1930-1931

<sup>2</sup> Fellow of the John Simon Guggenheim Memorial Foundation



The capsule and its clamp are shown in Figure 1. In making the capsule, *A*, a ring 1 cm wide is cut from celluloid tubing<sup>3</sup> 2.5 cm in diameter with a wall thickness of 1 mm. To this a cover of sheet celluloid, *B*, is cemented by acetone. The shape of the capsule is made conveniently oval by compressing the ring as the cover is cemented on. A tube 3 mm in diameter is cemented into a hole bored in the rim, for

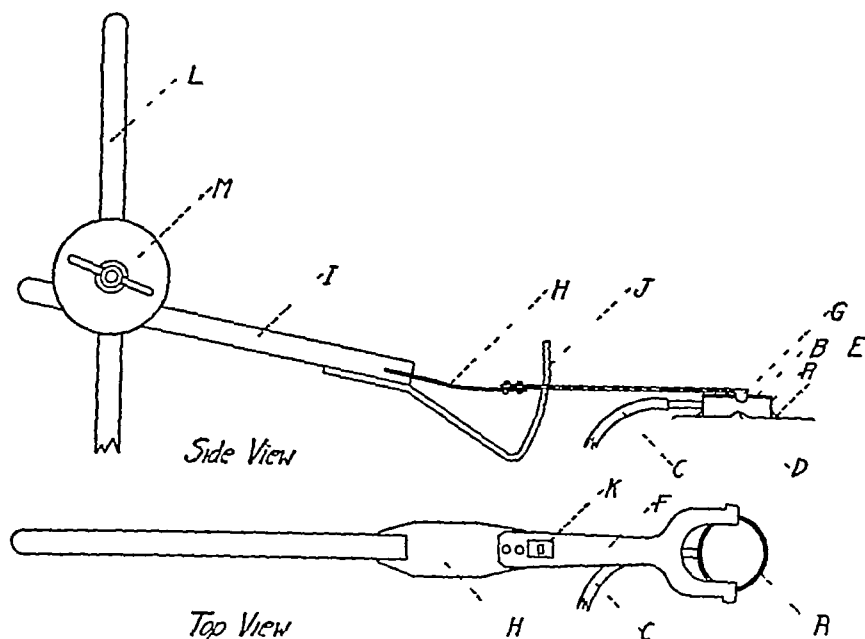


FIG 1 CAPSULE AND COUNTERWEIGHTING CLAMP USED FOR DETERMINATION OF VENOUS PRESSURE

For description, see text

communication with the pressure apparatus through the rubber tube, *C*. While the capsule is drying it is so placed as to be freely open to the air since acetone vapor lessens the transparency of the celluloid top. The advantages of celluloid as compared with glass are not only its unbreakability and the easy manufacture of new capsules but the fact that notches, *D*, can be cut in it to fit any conformation of veins.

#### *Observation of low venous pressures*

For the true reading of venous pressures the vein chosen must be separated from a firm underlying bony support by as little intervening tissue as possible. The capsule was so placed that the best portion of the vein chosen for observation was in the middle of the inclosed area. The capsule was cemented to the skin by a rather thick solution of collodion.

<sup>3</sup> Celluloid tubing of different sizes can be procured from Deutsche Celluloid-fabrik, Eulenburg, Sachsen, Germany. Other laboratory uses for these celluloid products are suggested by Krogh and Lange, 1930.

For low pressures a single coat and a drying time of ten minutes were found sufficient. The capsule was easily held in place during drying by the clamp to be described below. For pressures up to 30 or 40 cm of water a water manometer can be used. Pressure was applied by a thick-walled rubber bulb without valves. Compression was well-controlled by a screw clamp between the jaws of which the bulb was supported. Removal of the capsule at the end of the observations was effected by the aid of ether and alcohol which, however, was not allowed to touch the transparent top of the capsule.

The end point used in our determinations was not the mere collapse of the vein for it was difficult to tell when this was complete. When the skin was sufficiently transparent the end point was taken at the level where the thin blue line marking a very small amount of blood flowing in the flattened vein first reappeared after it had been driven out by a somewhat higher pressure. By a slight movement of the compressing screw up and down or, more effectively, by sharp pinches on the tube leading to the capsule small quick changes in pressure of about 1 to 1.5 cm were made. As the vein was watched during this manoeuvre, usually with oblique lighting, the disappearance and reappearance of this last thin stream of blood could be determined with considerable accuracy. If the tissues were less transparent the to and fro movement of the wall of the vein as distinct from the movement of the whole area of skin under the capsule was used as a criterion.

The validity of these determinations of relatively low pressure was tested by putting various pressures on the upper arm through an armlet with a rubber bag of ample size, 50 × 15 cm. Such an application of pressure is well known to raise the pressure of the blood in the veins below the armlet to a height approximately equal to the pressure applied. In a long series of experiments in which it was necessary to know venous pressures (Krogh, Landis and Turner, 1932) armlet pressures from 15 to 30 cm of water were checked many times and venous pressures in the hand veins found to correspond with great fidelity. The variation from the armlet pressure was rarely more than 1 cm.

#### *Observations of high venous pressures*

For determining high venous pressures such as those found in the veins of the feet during sitting or standing some modifications of procedure were made. In cementing on the capsule it was necessary to use two or three coats of collodion and to allow a much longer drying time, fully a half hour. A mercury manometer was used in connection with a large rubber bulb which was compressed by a screw clamp as before. The same technic for determining the end point was used as for low pressures though the zone of error was probably wider.

When pressure within the capsule is 30 mm Hg or more there is usually obvious distortion of the skin within and around the capsule,

though to a degree varying in different subjects and situations according to the looseness of the skin and underlying tissues. This cupping of the skin tends to facilitate the passage of blood under the capsule and thus to necessitate for obliteration of the flow a capsular pressure higher than the true venous pressure. To prevent error due to this cause the clamp shown in top and side views in Figure 1 was devised. This was applied to the capsule with a pressure approximately equal to the expected venous pressure thus holding the capsule down and preventing skin deformation. Blood flow was not affected because of the notches in the capsule. The clamp was made and graduated thus. A brass section, *F*, was made with jaws of a proper size to fit the capsule and with small vertical pieces, *G*, at the ends of the jaws to prevent lateral movement on the capsule. Other small vertical pieces at right angles to *G*, not shown in the figure, made the line of pressure on the capsule precise. The section *F* was securely soldered and fastened by small bolts to a thin steel spring, *H*, which in turn was soldered fast into the handle, *I*. The pressure of the clamp upon the capsule was obviously dependent upon the degree to which the spring *H* was bent. This bending was measured by a graduated brass arc, *J*, which was attached to the handle as shown and which passed through a hole, *K*, in the brass section *F*. The clamp was borne by a stand, *L*, with a heavy base and its adjustment was easily controlled by a universal clamp, *M*. The total length of the clamp was 27 cm.

In graduating the clamp its position was inverted and weights from 100 to 600 grams, covering the range of probable need, were hung from the brass jaws along the line where pressure was usually exerted on the capsule. The position of the spring for each additional hundred grams of weight was indicated upon the brass arc. To determine the weight to be placed upon the capsule the probable venous pressure is estimated from the comparative levels of the vein in question and the heart, and such a weighting from the spring is provided as will counterbalance the necessary pressure within the capsule and maintain its natural relation to the skin. Suppose, for example, that the vein is 60 cm. below the heart, the venous pressure then may not vary greatly from this, let us say 60 to 65 cm. water pressure (44 to 48 mm. Hg). The surface of an oval capsule with an average diameter of 23 mm. will be slightly less than 4.15 sq. cm. The weighting needed in this case to prevent deformation of the skin will be  $62.5 \times 4.15$  or about 260 grams. From the size of the scale on the clamp it is possible to apply pressures with an error of about 10 grams, a degree of accuracy entirely adequate. For a few pressures suitable weightings for a capsule of the size mentioned would be as follows

20 mm Hg venous pressure,	110 grams
40 mm Hg venous pressure,	225 grams
60 mm Hg venous pressure,	335 grams
80 mm Hg venous pressure,	450 grams

The validity of the determinations at the higher pressures was tested by putting the pressures indicated in Table 1 on the upper arm by an

TABLE 1

*Test for importance of counterweighting with use of celluloid capsule at high venous pressures  
For discussion see text*

Armlet pressure	Counterweight by clamp	Venous pressure as determined
mm. Hg	grams	mm. Hg
20	120	20
20	0	20
40	240	39 41
40	0	43 40
60	360	59, 60
60	0	62, 66
80	480	80
80	0	100, 94

armlet. The hand vein to be observed was kept at heart height. The pressure was applied in each case five minutes before the determination of venous pressure was made in order to give venous pressure time to come into equilibrium with armlet pressure. Counterweighting was employed as indicated, calculated from the size of the capsule and the expected venous pressure. After a determination was made at each pressure and while the intracapsular pressure remained on, the weighting clamp was gently removed. Cupping of the skin was seen as the pressure of the clamp was taken off. The vein was watched carefully for the entrance or lack of entrance of blood. If blood was seen to enter the vein, additional pressure was applied to collapse the vein again completely. In other words a measurement was made of the error which would have occurred had there been no counterweighting in the first determination. The pressures given in the table are single determinations, not averages, and are all from one subject. It will be seen that for 20 mm Hg there was no need of counterweighting for 40 mm it perhaps was not required though it was clearly needed for another subject at this pressure, but for the higher pressures of 60 and 80 mm Hg there was a large and variable error without the counterweight. The importance of counterweighting for the accurate determination of the higher venous pressures is thus clearly seen. It is safer to use it as soon as skin distortion is apparent.

Fogging of the capsule is a frequent difficulty with any attached capsule when observations extend over a considerable period. It is due to the condensation of water vapor on the under side of the top of the capsule and was avoided by keeping the capsule warm. A light pad of cotton wool applied between determinations was usually sufficient but in

more persistent fogging a silver disc heated in a flame to a suitable temperature was laid gently upon the capsule until the fog was dissipated

### SUMMARY

A celluloid capsule for the determination of venous pressures is described. This is very easily made and notches can be cut so that it will fit any arrangement of veins. It is cemented to the skin with collodion. When with high venous pressures the necessarily high intracapsular pressure would cause distortion of the skin and erroneous readings, the error can be avoided by the use of a counterweighting clamp, also described. Such a counterweight is usually needed for the measurement of venous pressures above 30 cm. water pressure.

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## CUTANEOUS RESPIRATION IN MAN

### IV THE RATE OF CARBON DIOXIDE ELIMINATION AND OXYGEN ABSORPTION IN NORMAL SUBJECTS<sup>1</sup>

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Shaw and his associates (1, 2) demonstrated that the rate of cutaneous respiration in man is influenced principally by the temperature and humidity of the air in contact with the skin and by individual characteristics of different subjects. The present investigation was undertaken to establish the range of carbon dioxide elimination and oxygen absorption through the skin in normal individuals of various ages under controlled conditions of temperature and relative humidity.

#### METHOD OF STUDY

The apparatus and technical procedure were essentially the same as those employed by Shaw and Messer (2) in studying the effect of temperature on the rate of cutaneous respiratory exchange. The subject lay on a bed with the entire arm in a glass plethysmograph filled with room air kept at the saturation point by means of a moist woolen stocking worn on the arm. The temperature within the plethysmograph varied between 26° C and 31° C in different experiments but was maintained practically constant during individual experiments by regulating the temperature of the room. All experiments were made with the room temperature between 20° C and 26° C.

After a preliminary mixing period of 20 minutes, a sample of gas was withdrawn from the plethysmograph. Three hours later a second sample was collected. The change in percentage concentration of carbon dioxide and oxygen multiplied by the total volume of gas in the system gave the volume of carbon dioxide or oxygen which had been gained or lost. All quantities were corrected to standard conditions of temperature and barometric pressure, and the rates of carbon dioxide excretion and oxygen absorption were expressed in terms of cubic centimeters per hour per square meter of skin surface. In order that the results obtained in different individuals should be comparable, all final quantities were transposed by interpolation to the value they would have had if the

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<sup>1</sup> This investigation was aided by a grant from the DeLamar Mobile Research Fund of Harvard University.

temperature of the air in the plethysmograph had been 27° C. This was done in the following manner. In each subject on whom three or more measurements of the rate of cutaneous respiration were made, the rates of carbon dioxide elimination and oxygen absorption in each experiment were plotted against the temperature of the air in the plethysmograph during that observation (Figure 1). Average straight lines were then con-

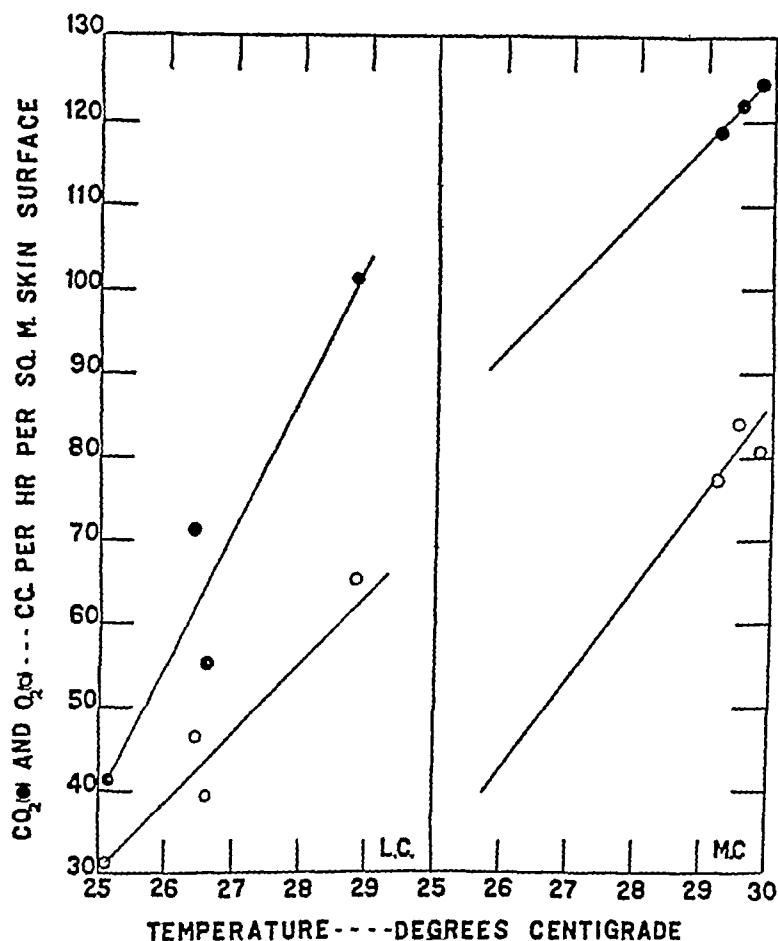


FIG 1 ILLUSTRATES THE METHOD FOR TRANSPOSING THE OBSERVED VALUES FOR CUTANEOUS RESPIRATION TO THEIR VALUES AT 27° C

Each charted point represents the rate of carbon dioxide elimination or oxygen absorption observed at the indicated temperature. The data obtained in two patients are presented and demonstrate the individual variations in the effect of temperature on cutaneous gas exchange in normal subjects.

constructed from the charted points. Shaw and Messer (2) have demonstrated that at temperatures below 34° C the rate of cutaneous respiration varies directly with the temperature of the air in contact with the skin. The rate of gas exchange through the skin at 27° C in our subjects,

therefore, was indicated by the height of the constructed lines at 27° C. In those subjects on whom only two measurements of cutaneous respiration were made, the results were transposed to their value at 27° C by using the average correction per degree rise in temperature for the entire group.

The rate of cutaneous respiration was measured on three or more occasions in the majority of the subjects, the observations on each individual being made on successive days whenever possible. In 20 subjects, two or more experiments were made under practically identical conditions of temperature. In four individuals, the rate of cutaneous respiration in the two arms was compared and in three, measurements were repeated at intervals over a period of two to several months. All observations were made between November 1, 1930 and June 2, 1931.

In 17 subjects, the rate of oxygen absorption through the lungs was determined during the estimation of the rate of cutaneous respiration.

## RESULTS

One hundred and twenty-one measurements of the rate of cutaneous respiration were made in 38 individuals between the ages of 15 and 75 years. Twelve of the subjects were females and 26 were males. The arterial blood pressure was within the limits of normal in all, and none presented lesions of the skin or evidence of respiratory, circulatory or metabolic disturbances. The erythrocyte count and hemoglobin content of the blood were within the limits of normal in all. A few of the elderly individuals showed slight to advanced sclerosis of the peripheral arteries.

*The range of cutaneous respiration in normal subjects.* A summary of the observations on each subject together with the maximum, minimum and average values recorded for the entire group are presented in Table 1. The rate of carbon dioxide elimination per hour per square meter of skin surface at 27° C. varied in different individuals from 58 cc. to 169 cc. with an average value of 120 cc. The rate of oxygen absorption per hour per square meter of skin surface at 27° C. averaged 88 cc. and varied in different individuals from 40 cc. to 146 cc. The respiratory quotient of cutaneous gas exchange at 27° C. averaged 1.4 and varied between 1.1 and 2.0.

Frequency distribution diagrams of the values for carbon dioxide elimination, oxygen absorption and respiratory quotient are presented in Figures 2 and 3. In 31 of the 38 subjects, the rate of carbon dioxide elimination varied between 100 cc. and 150 cc. per hour per square meter of skin surface, while in 29 individuals the rate of oxygen absorption varied between 60 cc. and 120 cc. per hour per square meter of skin surface. In 26 subjects, the respiratory quotient was between 1.2 and 1.6.

*Variations in the effect of temperature on the rate of cutaneous respiration.* The increase in carbon dioxide elimination with each degree rise in



TABLE 1  
*Cutaneous respiration in normal subjects at 27° C*

Subject	Sex	Age	CO <sub>2</sub> excreted		O <sub>2</sub> absorbed		CO <sub>2</sub> / O <sub>2</sub>	Temperature in plethysmograph		
			Per hour per square meter	Increase per degree rise in temperature†	Per hour per square meter	Increase per degree rise in temperature†		Experi- ment 1	Experi- ment 2	Experi- ment 3
		<i>years</i>	<i>cc</i>	<i>cc</i>	<i>cc</i>	<i>cc</i>		<i>° C</i>	<i>° C</i>	<i>° C</i>
1	F	15	117		74		1.6	29.0	29.0	27.5
2	M	15	118		82		1.4	26.3	27.4	
3	M	16	128		63		2.0	29.8	28.2	
4	F	17	134		87		1.5	29.9	30.0	
5	F	17	129		100		1.3	27.8	29.0	
6	M	19	142	4	96	5	1.5	29.2	27.6	28.9
7	M	19	143		116		1.2	30.8	29.4	
8	M	20	130	13	91	12	1.4	29.3	28.5	30.2
9	F	22	140	9	100	8	1.4	28.1	29.6	26.9
10	F	22	169	16	146	17	1.2	27.5	24.3	28.6
11	F	22	140	16	121	18	1.2	26.7	27.6	26.4
12	M	23	100	2	59	3	1.7	27.5	27.9	
13	F	24	93	1	64	2	1.5	29.2	29.9	29.5
14	M	24	107	4	84	4	1.3	29.0	28.4	28.2
15	M	24	103		85		1.2	30.0	28.8	29.1
16	M	24	101		76		1.3	26.5	26.6	28.4
17	F	24	131	7	119	7	1.1	28.1	30.4	27.2
18	F	24	143	18	115	16	1.2	28.3	27.6	25.9
19	M	24	156		138		1.1	29.6	28.4	27.7
20	M	25	147	5	110	4	1.3	29.8	28.0	28.6
21*	M	26	58	5	40	4	1.5	26.7	27.7	28.8
22	F	30	104	6	75	8	1.4	27.9	27.2	28.0
23	M	30	135	2	89	7	1.5	29.2	27.6	28.6
24	M	33	129	11	111	6	1.2	27.0	28.1	
25	M	35	129	7	77	7	1.7	29.1	30.0	
26	M	36	139	3	125	4	1.1	30.0	30.2	29.3
27	M	44	84	8	54	2	1.6	31.4	30.3	28.9
28	F	45	127	7	83	6	1.5	27.2	28.6	
29†	M	48	72	15	48	9	1.5	26.6	26.4	25.1
30†	M	50	106	5	55	7	1.9	29.4	29.7	
31†	M	50	120	14	65	17	1.9	28.9	30.3	
32	F	51	136	10	118	15	1.2	27.5	26.2	27.3
33†	M	53	101	6	81	6	1.2	27.8	29.6	28.3
34	M	57	109	5	60	7	1.8	29.2	29.8	29.5
35	M	61	124	6	75	5	1.7	28.4	28.4	29.5
36†	M	64	117	14	96	5	1.2	27.8	27.7	
37†	M	70	118		81		1.5	26.3	29.0	
38†	M	75	95	11	81	8	1.2	28.5	28.6	
Maximum value			169	18	146	18	2.0			
Minimum value			58	1	40	2	1.1			
Average value			120	8	88	8	1.4			

\* From Shaw and Messer (2)

† Subjects having moderate or advanced arteriosclerosis

‡ The increase in the rate of carbon dioxide elimination and oxygen absorption per degree rise in the temperature of the air in contact with the skin is given

temperature of the air in contact with the skin averaged 8 cc. per hour per square meter of skin surface and varied in different subjects from 1 cc. to 18 cc. (Table 1) The increase in oxygen absorption with each degree rise

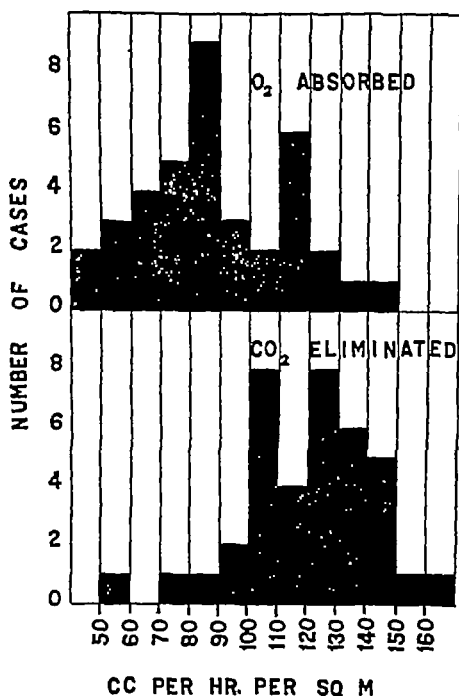


FIG 2 FREQUENCY DISTRIBUTION DIAGRAMS OF THE VALUES FOR CARBON DIOXIDE ELIMINATION AND OXYGEN ABSORPTION AT 27° C IN NORMAL SUBJECTS

in temperature of the air in contact with the skin varied in different subjects from 2 cc. to 18 cc. with an average value of 8 cc. per hour per square meter of skin surface.

*Variations in repeated measurements of the rate of cutaneous respiration in the same individual* In those subjects in whom two or more measure

for those subjects in whom three or more measurements of the rate of cutaneous respiration were made In those subjects on whom only two measurements were made, the results were transposed to their value at 27° C by using the average correction per degree rise in temperature for the entire group

ments of cutaneous respiration were made under practically identical conditions of temperature, small but significant variations were recorded (Table 2). The average variation in the amount of carbon dioxide eliminated was  $\pm 3$  cc per hour per square meter of skin surface, and the maximum variation was  $\pm 10$  cc. The average variation in the amount of oxygen absorbed was  $\pm 5$  cc per hour per square meter of skin surface, and the maximum variation was  $\pm 15$  cc. The average variation in the respiratory quotient was  $\pm 0.1$ , and the maximum variation was  $\pm 0.3$ .

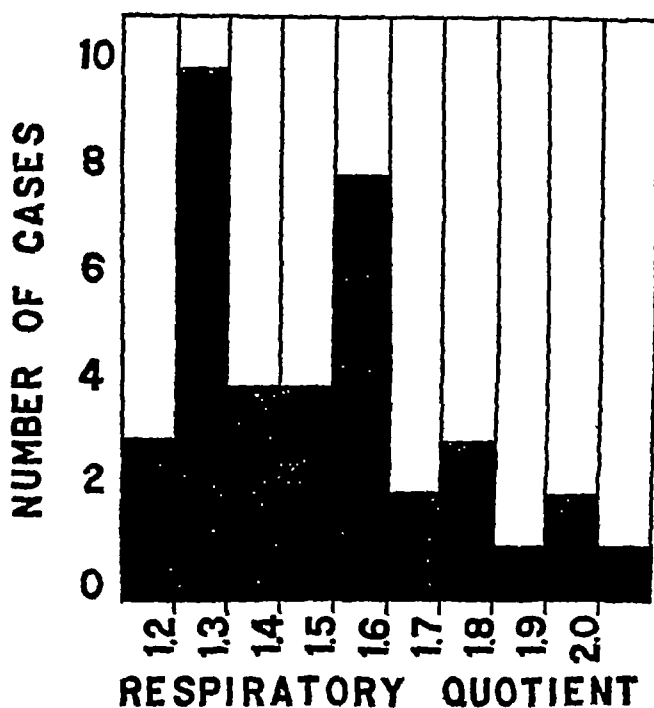


FIG 3 FREQUENCY DISTRIBUTION DIAGRAM OF THE VALUES FOR RESPIRATORY QUOTIENT AT 27° C IN NORMAL SUBJECTS

*Rate of cutaneous respiration in the two arms* No constant difference was observed in the rate of cutaneous respiration in the two arms (Table 3)

*Effect of season on the rate of cutaneous respiration* In the subjects on whom measurements of cutaneous respiration were repeated at intervals over a period of two to eight months, no evidence of seasonal variations in the rate of carbon dioxide excretion and oxygen absorption was observed (Table 4)

*Effect of sex on the rate of cutaneous respiration* No constant difference in the rate of cutaneous respiration was observed in the two sexes (Table 1)

*Effect of age on the rate of cutaneous respiration* The rate of carbon dioxide elimination and oxygen absorption tended to be greater in

individuals below the age of 40 years than in those above that age (Table 5) In the subjects over the age of 40 years, the rate of cutaneous respiration tended to be lower in individuals having moderate or advanced

TABLE 2

*Variations in the rate of cutaneous respiration under practically identical conditions of temperature\* and humidity*

Subject	Variation in CO <sub>2</sub> excreted	Variation in O <sub>2</sub> absorbed	Variation in respiratory quotient	Temperature in plethysmograph
	<i>cc per hour per square meter</i>	<i>cc per hour per square meter</i>		<i>C</i>
1	± 1	± 4	± 0.1	29.0
4	± 3	± 15	± 0.2	30.0
6†	± 6	± 9	± 0.1	30.0
11	± 3	± 6	± 0.0	26.6
12	± 0	± 2	± 0.0	27.7
13†	± 0	± 13	± 0.3	29.5
14	± 1	± 2	± 0.0	28.3
15	± 1	± 0	± 0.0	29.0
16	± 10	± 8	± 0.0	26.6
22	± 3	± 13	± 0.3	28.0
23†	± 3	± 0	± 0.0	29.8
26	± 0	± 8	± 0.1	30.1
29	± 8	± 4	± 0.1	26.5
30	± 5	± 1	± 0.0	29.6
32	± 7	± 4	± 0.0	27.4
33	± 3	± 0	± 0.0	28.1
34†	± 3	± 3	± 0.1	29.5
35	± 7	± 4	± 0.0	28.4
36	± 2	± 1	± 0.0	27.8
38	± 1	± 10	± 0.1	28.6
Average	± 3	± 5	± 0.1	

\* The maximum difference in temperature in the plethysmograph during the two (or three) experiments on one subject was 0.5° C. The temperatures given in the table represent the average for the two (or three) measurements on each individual.

† Indicates that the figures given are for the maximum variations observed in three experiments. All other figures are for the variations observed in two experiments.

‡ Indicates that the interval between the collection of samples was two hours instead of three.

generalized arteriosclerosis than in those presenting slight or no evidence of arteriosclerosis (Table 6). These relations were not exact in individual instances.

*Relation between the rate of respiratory exchange through the lungs and the rate of cutaneous respiration.* In 17 subjects, the average amount of oxygen absorbed through the lungs was 7.78 liters per hour per square meter of body surface. At 32° C, the approximate normal temperature

of the skin, the average calculated amount of oxygen absorbed through the skin in these individuals was 152 cc per hour per square meter, or 1.9 per cent of that absorbed through the lungs. In individual subjects no precise relationship was observed between oxygen absorption through the lungs and oxygen absorption through the skin.

TABLE 3

*Comparisons of the rate of cutaneous respiration in the two arms \**

Sub ject	CO <sub>2</sub> excreted		O <sub>2</sub> absorbed		Remarks
	Right arm	Left arm	Right arm	Left arm	
6	cc 146	cc. 139	cc 98	cc 95	Values for right arm are the average of five experiments, those for the left arm the average of seven measurements
9	135	142	87	105	Values for right arm are the average of three experiments, those for the left arm the average of six measurements
22	101	106	90	66	Values for right arm are the results of one experiment, those for the left arm the average of two measurements
23	143	129	94	84	Values for right arm are the average of three experiments, those for the left arm the average of four measurements

\* Calculated in cubic centimeters per hour per square meter of skin surface at 27° C

If it is assumed that the average quotient of respiratory exchange through the lungs in the above subjects was 0.82, then the average calculated amount of carbon dioxide excreted through the lungs was 6.37 liters per hour per square meter of body surface. At 32° C the average amount of carbon dioxide excreted through the skin in these individuals was 175 cc per hour per square meter, or 2.7 per cent of that excreted through the lungs. In individual subjects no precise relationship was observed between the amount of carbon dioxide eliminated through the lungs and the amount excreted through the skin.

## DISCUSSION

*Mechanism of cutaneous respiration* The total gaseous exchange through the skin is the result of two distinct processes (1) the metabolism of the skin, and (2) the passage of carbon dioxide out of the blood by diffusion through the skin (1). The fact that the respiratory quotient is always above unity, with an average value of 1.4 in our subjects, indicates

TABLE 4

*The effect of season on the rate of cutaneous respiration \**

Subject	Date	CO <sub>2</sub> excreted	O <sub>2</sub> absorbed	CO <sub>2</sub> /O <sub>2</sub>	Temperature in plethysmograph
6	October 30, 1930	cc	cc.		C
	October 31 1930	147	94	1.6	29.2
	November 3, 1930	157	116	1.4	27.6
	November 3, 1930	137	95	1.4	28.9
	December 11 1930	154	105	1.5	29.4
	March 2 1931†	155	83	1.9	30.9
	April 15 1931†	142	96	1.5	30.0
	April 25 1931†	114	82	1.4	28.3
	April 28 1931†	134	88	1.5	28.4
	May 14 1931†	132	83	1.6	28.1
	May 22 1931†	153	113	1.4	30.0
	May 25 1931†	127	84	1.5	27.5
	May 29 1931†	151	113	1.3	30.7
	November 12 1930	164	131	1.3	28.1
9	November 20 1930	142	116	1.2	29.6
	November 29 1930	125	107	1.2	26.9
	February 2 1931	146	75	2.0	29.8
	March 4, 1931†	130	75	1.7	28.8
	April 14, 1931†	135	80	1.7	28.6
	May 18 1931†	122	85	1.4	27.4
	May 28 1931†	154	114	1.4	29.4
	June 2, 1931†	140	107	1.3	29.0
	April 1 1931†	124	84	1.5	29.2
	April 27, 1931†	128	90	1.4	27.6
23	April 29 1931†	128	75	1.7	28.6
	May 19, 1931†	140	88	1.6	29.8
	May 20 1931†	156	103	1.5	30.6
	May 21, 1931†	132	92	1.4	28.5
	May 26 1931†	134	88	1.5	29.7

\* Calculated in cubic centimeters per hour per square meter of skin surface at 27° C

† Indicates that the interval between the collection of samples was two hours instead of three.

TABLE 5

*The effect of age on the rate of cutaneous respiration \**

Age Group	Num ber of cases	Rate of CO <sub>2</sub> excretion			Rate of O <sub>2</sub> absorption			CO <sub>2</sub> /O <sub>2</sub>		
		Maxi mum	Mini mum	Aver age	Maxi mum	Mini mum	Aver age	Maxi mum	Mini mum	Aver age
15-39	26	cc. 169	cc. 58	cc. 126	cc. 146	cc. 40	cc. 94	2.0	1.1	1.4
40-75	12	136	72	109	118	54	75	1.9	1.2	1.5

\* Calculated in cubic centimeters per hour per square meter of skin surface at 27° C

TABLE 6

*The effect of arteriosclerosis on the rate of cutaneous respiration \* in subjects 40 years of age or over*

Subjects	Number of cases	Rate of CO <sub>2</sub> excretion			Rate of O <sub>2</sub> absorption			CO <sub>2</sub> /O <sub>2</sub>		
		Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum	Average
With slight or no arteriosclerosis	5	cc 136	cc 84	cc 115	cc 118	cc 54	cc 78	1 8	1 2	1 6
With moderate or advanced arteriosclerosis	7	124	72	104	96	48	72	1 9	1 2	1 5

\* Calculated in cubic centimeters per hour per square meter of skin surface at 27° C

that the carbon dioxide eliminated cannot originate exclusively from the metabolic processes of the skin and that diffusion of this gas from the blood through the skin is of considerable importance. In contrast to this dual source of carbon dioxide, the oxygen absorbed by the skin probably is utilized entirely in tissue oxidation, no part of it passing into the blood by diffusion through the tissues (3).

*Range of cutaneous respiration in normal subjects* Although the observations of earlier investigators (1) had led us to expect a relatively wide range of normal values for carbon dioxide elimination and oxygen absorption through the skin of different individuals, we did not anticipate the extreme variations actually observed. Whether the differences are due mainly to individual variations in the extent to which the metabolic requirements of the skin are supplied by the exchange of gases between the tissues and the air or to variations in the actual metabolic rate of the skin of different subjects cannot be stated with certainty. It seems doubtful, however, that the metabolic rate of the skin is subject to much greater individual variations than the total metabolism of the body. On the other hand, it is conceivable that, as the result of individual differences in blood supply to the skin, tension of oxygen and carbon dioxide in the blood, and other factors, the amount of oxygen supplied to the skin by the blood and the amount of carbon dioxide removed may vary widely. Variations of this kind produce reciprocal changes in the rate of oxygen absorption and carbon dioxide elimination through the skin (3). We are inclined to believe, therefore, that the wide range of normal values is due in large part to individual differences in the extent to which the exchange of gases between the blood and the skin meets the needs of cutaneous metabolism, rather than to differences in the metabolic rate of the skin.

It has been suggested (1) that the relatively wide variations observed in the respiratory quotient of cutaneous respiration are due mainly to the fact that carbon dioxide diffuses through living tissues more rapidly than does oxygen, and so slight changes in the carbon dioxide tension of the cutaneous blood cause disproportionate changes in the respiratory quotient. If this hypothesis were correct, the rate of oxygen absorption through the skin should be more constant than the rate of carbon dioxide elimination, provided, of course, that the respiratory quotient of the skin itself remained constant. Inspection of the data in Tables 2 and 4 reveals that variations in the rate of oxygen absorption were as great as the variations in the rate of carbon dioxide elimination and were responsible for fluctuations in the respiratory quotient as frequently as were changes in the rate of carbon dioxide elimination. It seems, therefore, that either the extra metabolic factors influencing the rate of oxygen absorption through the skin vary as greatly as do the factors affecting the rate of carbon dioxide elimination or that the observed fluctuations in respiratory quotient reflect actual changes in the metabolic processes of the skin. Although no final decision can be made at present, it seems doubtful that changes in the intrinsic metabolism of the skin are the responsible factor.

*Variations in the effect of temperature on the rate of cutaneous respiration*  
The rate of cutaneous respiration increases as the temperature of the air in contact with the skin rises, but the relationship between temperature and the rate of cutaneous gas exchange varies widely in different individuals (Table 1). Because of the evidence that the oxygen absorbed by the skin is utilized entirely in tissue oxidation (3), we believe that the accelerated rate of gas exchange accompanying increased temperature is due principally to a rise in the metabolic rate of the skin. It is probable, however, that the metabolic rate of the skin is affected by changes in temperature in a more or less equal degree in all normal subjects. The blood supply to the skin also increases with higher temperatures, and the improved circulation undoubtedly supplies part of the increased metabolic needs of the tissues. Because of differences in the sensitivity of the autonomic nervous system, the degree to which the blood supply is altered in response to changes in temperature may vary within wide limits in different individuals. Variations are therefore to be expected in the extent to which the circulating blood supplies the increased metabolic needs of the skin at higher temperatures. We believe that differences of this kind, rather than differences in the effect of temperature on the metabolic rate of the skin, are responsible for the observed variations in the effect of temperature on the rate of cutaneous respiration.

*Variations in repeated measurements of the rate of cutaneous respiration in the same individual*  
In different individuals, variations in carbon dioxide elimination, oxygen absorption and respiratory quotient probably



are due in large part to constitutional factors inherent in the subjects. The variations observed in repeated measurements on the same subjects under identical conditions of temperature and humidity (Table 2), however, are due to unrecognized changes in the experimental conditions or to physiological variations in the patients. The analytical error for the measurement of carbon dioxide excretion in our system was about  $\pm 1.3$  cc per hour per square meter of skin surface, and for oxygen absorption about  $\pm 2.6$  cc per hour. Even though allowance is made for this error, it is still evident that in many of the experiments summarized in Table 2, some factor or group of factors capable of producing changes in the rate of cutaneous respiration and in the respiratory quotient remained uncontrolled. In view of the evidence that fluctuations in the tension of carbon dioxide and oxygen in the blood cause changes in the rate of cutaneous respiration (3), it seems probable that physiological variations in the tension of the blood gases constitute the principal factor responsible for the differences observed in repeated measurements under identical conditions of temperature and humidity.

*Effect of season on the rate of cutaneous respiration* Shaw, Messer and Weiss (1) observed a distinct tendency for the rate of carbon dioxide elimination through the skin to increase during the colder months of the year. Our studies, on the other hand, have revealed no evidence of seasonal variations in the rate of cutaneous respiration (Table 4). The experiments of Shaw, Messer and Weiss were made before the introduction of measures to control the humidity of the air within the plethysmograph, while our observations were made with the air in the system at the saturation point. This difference in technique may account for the discrepancy in results.

*Effect of age on the rate of cutaneous respiration* The tendency for subjects above the age of 40 years to have a lower rate of cutaneous respiration than younger individuals (Tables 1 and 5) is of considerable interest. The fact that the diminution in rate tended to be more marked in those individuals having moderate or advanced generalized arteriosclerosis than in those having slight or no evidence of arteriosclerosis (Table 6) suggests that the decrease is related to involutionary changes in the skin.

Certain experiments of Shaw and Messer (3) indicate that diminished blood supply to the skin is attended by an increased rate of cutaneous gas exchange, provided that the metabolic rate of the skin remains unchanged. In other words, a decrease in the degree to which the circulating blood supplies the metabolic needs of the skin is compensated for by an increase in the extent to which those needs are met by direct exchange of carbon dioxide and oxygen through the skin. In view of this consideration, diminished blood supply to the skin cannot be responsible for the observed reduction in the rate of cutaneous respiration in subjects

above the age of 40 years. On the other hand, the evidence that the oxygen absorbed by the skin is utilized entirely in tissue oxidation (3), suggests that the reduced rate of cutaneous gas exchange is due to an actual decrease in the metabolic rate of the skin.

*Relation between the rate of respiratory exchange through the lungs and the rate of cutaneous respiration.* In our subjects, the cutaneous exchange of carbon dioxide and oxygen amounted to a much larger percentage of pulmonary exchange than observed by earlier investigators. Aubert (4) estimated that one half of one per cent of the expired carbon dioxide was eliminated through the skin. The data presented by Zuelzer (5) indicated that the average amount of oxygen absorbed by the skin was one per cent of the amount absorbed through the lungs. Because of errors inherent in the methods utilized by these observers, the results obtained are of doubtful value. Shaw, Messer and Weiss (1) calculated that in their two subjects the carbon dioxide eliminated through the skin amounted to one per cent of that given off through the lungs. In our observations on 17 subjects, the average cutaneous absorption of oxygen amounted to 1.9 per cent of pulmonary absorption, and the cutaneous elimination of carbon dioxide amounted to 2.7 per cent of pulmonary elimination. In view of the fact that cutaneous respiratory exchange results in part from the metabolism of the skin and in part from the diffusion of carbon dioxide through the skin, it is not surprising that, in individual subjects, no exact relationship was observed between the rate of cutaneous respiration and the rate of carbon dioxide elimination and oxygen absorption through the lungs.

### SUMMARY

1 Repeated measurements were made of the rate of carbon dioxide elimination and oxygen absorption through the skin in 38 normal subjects.

2 The rate of carbon dioxide elimination per hour per square meter of skin surface at 27° C. varied in different individuals from 58 cc. to 169 cc. with an average value of 120 cc.

3 The rate of oxygen absorption per hour per square meter of skin surface at 27° C. varied in different individuals from 40 cc. to 146 cc. with an average value of 88 cc.

4 The respiratory quotient of cutaneous gas exchange at 27° C. averaged 1.4 and varied between 1.1 and 2.0.

5 With each degree rise in the temperature of the air in contact with the skin, an average increase of 8 cc. per hour per square meter of skin surface was observed in both carbon dioxide elimination and oxygen absorption. The accelerated rate of gas exchange through the skin at higher temperatures probably is due principally to an increased rate of cutaneous metabolism.

6 No relationship was observed between the rate of cutaneous respiration and the sex of the subjects or the season of the year.

7 The rate of carbon dioxide elimination and oxygen absorption through the skin tended to be lower in subjects above the age of 40 years than in those below that age. The diminished rate of cutaneous respiration in subjects above the age of 40 years probably is due to a decreased metabolic rate of the skin.

8 In 17 subjects, the average amount of oxygen absorbed through the skin was 1.9 per cent of that absorbed through the lungs, and the average amount of carbon dioxide excreted through the skin was 2.7 per cent of that excreted through the lungs. In individual subjects no exact relationship was observed between the rate of respiratory exchange through the lungs and the rate of cutaneous respiration.

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## CUTANEOUS RESPIRATION IN MAN

### V THE RATE OF CARBON DIOXIDE ELIMINATION AND OXYGEN ABSORPTION IN SUBJECTS WITH DISEASES OF THE SKIN<sup>1</sup>

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A suitable method for measuring the rate of cutaneous respiration in man has been developed by Shaw and his associates (1, 2), and normal standards for carbon dioxide elimination and oxygen absorption have been established (3). The present communication deals with an investigation of the rate of cutaneous exchange of carbon dioxide and oxygen in subjects with various pathologic conditions of the skin.

#### METHOD OF STUDY

The apparatus and technical procedure were the same as those employed in preceding studies on the rate of cutaneous respiration in normal individuals (2, 3). In brief, the total amounts of carbon dioxide eliminated and oxygen absorbed through the skin of the entire arm in a period of three hours were measured, and the results were expressed in terms of cubic centimeters per hour per square meter of skin surface. The relative humidity of the air in the plethysmograph enclosing the arm was kept at the saturation point by means of a moist woolen stocking worn on the arm. The temperature of the air in contact with the skin varied between 26° C and 31° C in different experiments but was maintained practically constant during individual experiments by regulating the temperature of the room. All measurements were made with the room temperature between 20° C and 25° C. In order to compare the results in patients with skin diseases with the rate of cutaneous respiration in normal subjects, all final quantities were transposed by interpolation to the value they would have had if the temperature of the air in the plethysmograph had been 27° C. The manner of making this correction has been described previously (3).

#### RESULTS

The rate of cutaneous respiration was measured on three or more occasions in twelve subjects with pathologic conditions of the skin, the

<sup>1</sup> This investigation was aided by a grant from the DeLamar Mobile Research Fund of Harvard University.

observations on each individual being made on successive days whenever possible. Only patients presenting diffuse lesions involving a large part of the upper extremity were selected for the experiments. Five subjects had psoriasis, two had eczema, and one each had erythema multiforme, dermatitis venenata, scleroderma, exfoliative dermatitis, and seborrheic dermatitis. In two patients with psoriasis, additional measurements of the rate of cutaneous respiration were made after the lesions had disappeared. A summary of the observations is presented in Tables 1 and 2.

In general, the rate of cutaneous respiration tended to be somewhat elevated. In only one subject was the rate of carbon dioxide elimination lower than 130 cc per hour per square meter of skin surface, while in 25 of 38 normal individuals the rate was less than 130 cc per hour (3). In 6 of the 12 patients with skin disease the rate of carbon dioxide elimination was 150 cc per hour per square meter of skin surface or higher, while a rate of 150 cc or more was recorded in only two normal subjects. In three patients with skin disease the rate of carbon dioxide elimination was higher than in any of the normal subjects. The rate of carbon dioxide excretion tended to vary directly with the severity of the pathologic process, the two highest rates being observed in the two patients presenting the most diffuse lesions and the greatest degree of accompanying inflammation. The average rate of carbon dioxide elimination for the entire group of patients with skin disease was 162 cc per hour per square meter of skin surface, as compared with an average rate of 120 cc in normal subjects.

The effect of pathologic conditions of the skin on the rate of oxygen absorption was somewhat less evident than the effect on carbon dioxide elimination. In 8 of the 12 subjects the rate of oxygen absorption was 100 cc or more per hour per square meter of skin surface, while rates of this magnitude were recorded in only 12 of 38 normal subjects (3). In only one patient with skin disease, however, was the rate of oxygen absorption higher than observed in any normal subject. The relationship between the rate of oxygen absorption and the severity of the pathologic process was less apparent than in the case of carbon dioxide elimination. The average rate of oxygen absorption in the patients with skin disease was 110 cc per hour per square meter of skin surface, as compared with an average of 88 cc in normal subjects.

A tendency toward slightly elevated values for the respiratory quotient of cutaneous respiration was noted in the patients with pathologic conditions of the skin. In 5 of the 12 subjects the respiratory quotient was 1.6 or higher, while values of this magnitude were recorded in only 9 of 38 normal individuals (3). In only one patient with skin disease, however, was the respiratory quotient higher than the highest observed normal value. The average value for respiratory quotient in the patients with skin disease was 1.5 as compared with an average value of 1.4 in normal individuals.

TABLE 1

*The rate of cutaneous respiration in subjects with pathologic conditions of the skin*

Subject	Sex	Age	Diagnosis	CO <sub>2</sub> excreted*	O <sub>2</sub> absorbed*	Respiratory quotient	Temperature in plethysmograph		
							Experiment 1	Experiment 2	Experiment 3
		years		cc	cc		C	C	C
39	M	14	Seborrheic dermatitis, severe	266	192	1.4	29.8	26.6	29.2
40	M	40	Exfoliative dermatitis	225	107	2.1	27.3	28.8	28.2
41	F	30	Psoriasis moderately severe	179	139	1.3	29.2	29.8	29.6
42	M	58	Dermatitis venenata, moderately severe	165	100	1.7	27.4	27.2	28.6
43	M	32	Psoriasis, severe	157	90	1.7	30.0	28.8	27.3
44	F	18	Eczema mild	150	123	1.2	26.7	27.2	27.2
45	F	42	Scleroderma, moderately advanced	147	119	1.2	28.0	26.7	30.6
46	F	15	Eczema moderately severe	137	106	1.3	29.0	26.0	27.4
47	F	29	Psoriasis, moderately severe	134	83	1.6	27.7	29.4	29.0
48	F	49	Psoriasis mild	134	87	1.5	29.6	29.2	30.1
49	F	32	Erythema multiforme, subsiding	132	70	1.9	31.0	28.4	28.8
50	F	42	Psoriasis mild	118	101	1.3	26.2	28.0	29.3
Average				162	110	1.5			

\* Calculated in cubic centimeters per hour per square meter of skin surface at 27° C. Each figure for carbon dioxide elimination and oxygen absorption represents the corrected average rate.

TABLE 2

*The rate of cutaneous respiration before and after the disappearance of skin lesions*

Subject	Diagnosis	CO <sub>2</sub> excreted*		O <sub>2</sub> absorbed*		Respiratory quotient	
		Before	After	Before	After	Before	After
43	Psoriasis severe	157	131	90	81	1.8	1.6
47	Psoriasis, moderately severe	134	134	83	93	1.6	1.4

\* Calculated in cubic centimeters per hour per square meter of skin surface at 27° C. Each figure for carbon dioxide elimination and oxygen absorption represents the corrected average rate.

The temperature in the plethysmograph during the measurements made before disappearance of the skin lesions is given in Table 1. The temperature in the plethysmograph during the measurements made after the disappearance of the lesions was as follows: subject 43, first experiment 28.1° C, second experiment 29.3° C. Subject 47, first experiment 26.0° C, second experiment 28.5° C.

In the two patients with psoriasis in whom further measurements of the rate of cutaneous respiration were made after the lesions had disappeared, no striking changes were observed (Table 2)

## DISCUSSION

Cutaneous respiration is the result of two distinct processes (1) the metabolism of the skin, and (2) the passage of carbon dioxide out of the blood by diffusion through the skin (1, 4) In contrast to the dual origin of the carbon dioxide eliminated through the skin, all of the oxygen absorbed by the skin probably is utilized in tissue oxidation (4)

The rate at which the skin absorbs oxygen from the air is influenced not only by the metabolic rate of the skin but also by the oxygen tension of the air and of the blood (4) With a constant oxygen tension of the air, the rate of oxygen absorption is increased by an increase in the metabolic rate of the skin or by a decrease in the oxygen tension of the blood In individuals with widespread lesions of the skin and accompanying inflammatory changes, a diminished oxygen tension of the blood would hardly be expected The tendency toward an increased rate of oxygen absorption in these subjects probably is due, therefore, to an increase in the metabolic rate of the skin

Increased cutaneous elimination of carbon dioxide may be due to an increase in the metabolic rate of the skin or to an increase in the amount of carbon dioxide escaping from the blood by diffusion through the skin An increase in the amount of carbon dioxide escaping from the blood by diffusion through the skin is dependent on an increase in the carbon dioxide tension of the blood Since, in individuals with skin disease, there is no apparent cause for an appreciable increase in the carbon dioxide tension of the blood, the increased rate of carbon dioxide elimination in these subjects is probably due to an increase in the metabolic rate of the skin

The tendency toward elevation of the respiratory quotient of cutaneous gas exchange in subjects with pathologic conditions of the skin could be the result of an actual qualitative change in skin metabolism On the other hand, if cutaneous metabolism remained qualitatively constant but increased in rate, the respiratory quotient would be elevated in the event that a larger part of the excess carbon dioxide were eliminated through the skin than by way of the blood That such an occurrence is possible is indicated by the following considerations The carbon dioxide tension of the air is practically zero, and the difference between this tension and the carbon dioxide tension of the skin is much greater than the difference between the carbon dioxide tension of the blood and of the skin (4) It is probable, therefore, that practically all of the carbon dioxide formed in the superficial layers of the skin is excreted directly into the air and that, of the carbon dioxide formed in the deeper layers, a larger part

is eliminated by excretion through the skin than by way of the blood stream. Similarly, in the event of increased production of carbon dioxide, all of this gas formed in the superficial layers of the skin would still be excreted directly into the air and a proportionately larger part of the excess formed in the deeper layers of the skin probably would be eliminated by cutaneous excretion. It is commonly assumed, furthermore, that carbon dioxide diffuses through living tissue at a greater rate than does oxygen. An increased tension of carbon dioxide in the tissues might therefore cause an appreciable increase in the rate of carbon dioxide elimination through the skin, while a corresponding decrease in the tension of oxygen in the tissues might have much less effect on the rate of cutaneous absorption of oxygen. Because of these considerations, we believe that the elevated respiratory quotient of cutaneous gas exchange in subjects with pathologic conditions of the skin results from the increased rate of cutaneous metabolism in these individuals rather than from qualitative changes in the metabolic processes of the skin.

The fact that no significant change in the rate of cutaneous respiration was recorded in the two subjects in whom additional measurements were made after the skin lesions had disappeared (Table 2) is not in harmony with the observed relationship between the rate of carbon dioxide elimination and the severity of the skin lesions. The reason for this conflict is not apparent.

The patient with scleroderma presented diffuse, board like infiltration of the skin of the entire upper extremity. It was rather surprising, therefore, that the rate of cutaneous respiration was as high as actually observed. It is quite possible that the vascular changes present in scleroderma interfere with the exchange of carbon dioxide and oxygen between the tissues and the blood, and that, in consequence, a larger proportion of gaseous exchange than normal takes place by diffusion between the tissues and the atmosphere in order to meet the metabolic needs of the skin. The fact that the average respiratory quotient in our patient was 1.2 indicates, however, that the metabolic processes of the skin were not the sole source of the carbon dioxide eliminated and that carbon dioxide was still passing out of the blood by diffusion through the skin.

## SUMMARY

1 Repeated measurements were made of the rate of cutaneous respiration in twelve subjects with pathologic conditions of the skin. Five patients had psoriasis, two had eczema, and one each had erythema multiforme, dermatitis venenata, scleroderma, exfoliative dermatitis, and seborrheic dermatitis.

2 The rate of cutaneous respiration tended to be elevated in these subjects.



3 The average rate of carbon dioxide elimination was 162 cc per hour per square meter of skin surface as compared with an average rate of 120 cc in normal subjects

4 The average rate of oxygen absorption was 110 cc per hour per square meter of skin surface as compared with an average rate of 88 cc in normal subjects

5 The average value for the respiratory quotient of cutaneous gas exchange was 1.5 as compared with an average value of 1.4 in normal individuals

6 The results of the investigation indicate that the metabolic rate of the skin is increased in subjects with widespread cutaneous lesions

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## CUTANEOUS RESPIRATION IN MAN

### VI THE EFFECT OF DRUGS ON THE RATE OF CARBON DIOXIDE ELIMINATION AND OXYGEN ABSORPTION<sup>1</sup>

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Cutaneous respiration is the result of two distinct processes (1) the passage of carbon dioxide out of the blood by diffusion through the skin, and (2) the intrinsic metabolism of the skin. The extent to which the metabolic requirements of the skin are supplied by cutaneous gas exchange depends on the relative tension of oxygen and carbon dioxide in the air and in the blood. With a constant oxygen tension of the air, the rate at which the skin absorbs oxygen from the air varies inversely with the oxygen tension of the blood (1). Similarly, there is evidence that, with a constant carbon dioxide tension of the air, the rate at which the skin excretes carbon dioxide into the air increases as the carbon dioxide tension of the blood rises (1). In addition, increased carbon dioxide tension of the blood probably accelerates the rate at which carbon dioxide passes out of the blood by diffusion through the skin. In view of these considerations, alterations in the blood supply to the skin would affect the rate of cutaneous respiration under normal atmospheric conditions if the changes in blood flow were accompanied by changes in the rate of cutaneous metabolism or in the tension of carbon dioxide and oxygen in the blood. Similarly, variations in the degree of activity of the sweat glands would influence the rate of cutaneous respiratory exchange if such variations were attended by changes in the rate of cutaneous metabolism or by alterations in blood flow accompanied by changes in the tension of the gases in the blood. This paper deals with the effect on the rate of cutaneous respiration of drugs capable of altering the blood supply to the skin or the degree of activity of the sweat glands.

#### METHOD OF STUDY

The apparatus and technical procedure were essentially the same as those employed in preceding studies (2, 3, 4) on the rate of cutaneous respiration in normal individuals and in subjects with pathologic conditions of the skin. Certain modifications, however, were introduced

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<sup>1</sup> This investigation was aided by a grant from the DeLamar Mobile Research Fund of Harvard University

The interval between the collection of samples was reduced to two hours in order that two successive experiments could be made on each subject. In each experiment the first sample of gas was collected after a preliminary mixing period, twenty minutes in duration. The subject's arm was removed from the plethysmograph between the two observation periods, and the apparatus was washed out with room air. The first experiment, in each instance, was a control observation, and the drug whose effect was being studied was administered at the end of the mixing period of the second experiment.

In brief, measurements were made of the total amounts of carbon dioxide eliminated and oxygen absorbed through the skin of the entire arm in two successive two-hour periods, the second of which immediately followed the administration of one of the drugs under investigation. The relative humidity of the air in contact with the skin was kept at the saturation point by means of a moist woolen stocking worn on the arm. The temperature of the air in the system varied between 26° C and 31° C in different experiments but was maintained practically constant during individual experiments by regulating the temperature of the room. All measurements were made with the room temperature between 20° C and 26° C. The results of each experiment were expressed in terms of cubic centimeters per hour per square meter of skin surface, and were transposed by interpolation to the value they would have had if the temperature of the air in the plethysmograph had been 27° C. The manner of making this correction has been described previously (3).

All drugs were administered by subcutaneous injection.

## RESULTS

Measurements were made of the effect of epinephrine, pituitary posterior lobe extract, histamine and pilocarpine on the rate of cutaneous respiration in three normal subjects. During a considerable part of the second observation period in each experiment the subjects presented symptoms and signs characteristic of the action of the drug which had been administered. Particular attention was paid to changes in the color of the skin. Epinephrine caused moderate pallor and pituitary posterior lobe extract, intense pallor. Histamine produced intense flushing of the face and slight flushing of the arms. Pilocarpine caused slight flushing and, in addition, profuse perspiration attended by a decided decrease in the temperature of the skin. None of the drugs produced constant changes in the rate of carbon dioxide elimination and oxygen absorption through the skin (Table 1).

TABLE 1  
*The effect of drugs on the rate of cutaneous respiration\**

Subject	Drug	CO <sub>2</sub> excreted			O <sub>2</sub> absorbed		
		Control period	After drug	Difference	Control period	After drug	Difference
9	Epinephrine hydrochloride, 1 cc.	140	139	- 1	107	104	- 3
23	Epinephrine hydrochloride, 1 cc.	132	143	+ 11	92	108	+ 16
6	Pituitary extract, 1 cc.	153	149	- 4	113	105	- 8
9	Pituitary extract, 1 cc.	154	146	- 8	114	120	+ 6
23	Pituitary extract, 1 cc.	156	156	0	103	115	+ 12
6	Histamine hydrochloride 0.001 gram	151	155	+ 4	113	104	- 9
23	Histamine hydrochloride 0.001 gram	134	122	- 12	88	92	+ 4
6	Pilocarpine hydrochloride, 0.008 gram	134	146	+ 12	88	69	- 19
6	Pilocarpine hydrochloride 0.008 gram	132	115	- 17	83	83	0
9	Pilocarpine hydrochloride 0.015 gram	122	132	+ 10	85	97	+ 12
23	Pilocarpine hydrochloride 0.008 gram	128	131	+ 3	75	86	+ 11
23	Pilocarpine hydrochloride 0.015 gram	140	158	+ 18	88	99	+ 11

\* Calculated in cubic centimeters per hour per square meter of skin surface at 27° C

## DISCUSSION

In an earlier investigation (3) repeated measurements were made of the rate of cutaneous respiration under practically identical conditions of temperature and relative humidity in a series of normal subjects. The average individual variation in the rate of carbon dioxide excretion was  $\pm 3$  cc. per hour per square meter of skin surface, and the greatest variation was  $\pm 10$  cc. The average individual variation in the rate of oxygen absorption was  $\pm 5$  cc. per hour per square meter of skin surface, and the greatest variation was  $\pm 15$  cc. Since the temperature of the air in the plethysmograph remained practically constant throughout each complete experiment in the present investigation, any change in the rate of carbon dioxide excretion should exceed 20 cc. per hour per square meter of skin surface in order to be significant. Similarly, changes in the rate of oxygen absorption of less than 30 cc. per hour per square meter of skin surface may be disregarded. The rate of cutaneous respiration, therefore, was not significantly affected by any of the drugs employed (Table 1).

The changes produced by the drugs in the color of the skin indicate that cutaneous blood flow had been altered. Although these changes

The interval between the collection of samples was reduced to two hours in order that two successive experiments could be made on each subject. In each experiment the first sample of gas was collected after a preliminary mixing period, twenty minutes in duration. The subject's arm was removed from the plethysmograph between the two observation periods, and the apparatus was washed out with room air. The first experiment, in each instance, was a control observation, and the drug whose effect was being studied was administered at the end of the mixing period of the second experiment.

In brief, measurements were made of the total amounts of carbon dioxide eliminated and oxygen absorbed through the skin of the entire arm in two successive two-hour periods, the second of which immediately followed the administration of one of the drugs under investigation. The relative humidity of the air in contact with the skin was kept at the saturation point by means of a moist woolen stocking worn on the arm. The temperature of the air in the system varied between 26° C and 31° C in different experiments but was maintained practically constant during individual experiments by regulating the temperature of the room. All measurements were made with the room temperature between 20° C and 26° C. The results of each experiment were expressed in terms of cubic centimeters per hour per square meter of skin surface, and were transposed by interpolation to the value they would have had if the temperature of the air in the plethysmograph had been 27° C. The manner of making this correction has been described previously (3).

All drugs were administered by subcutaneous injection.

## RESULTS

Measurements were made of the effect of epinephrine, pituitary posterior lobe extract, histamine and pilocarpine on the rate of cutaneous respiration in three normal subjects. During a considerable part of the second observation period in each experiment the subjects presented symptoms and signs characteristic of the action of the drug which had been administered. Particular attention was paid to changes in the color of the skin. Epinephrine caused moderate pallor and pituitary posterior lobe extract, intense pallor. Histamine produced intense flushing of the face and slight flushing of the arms. Pilocarpine caused slight flushing and, in addition, profuse perspiration attended by a decided decrease in the temperature of the skin. None of the drugs produced constant changes in the rate of carbon dioxide elimination and oxygen absorption through the skin (Table 1).

TABLE 1  
*The effect of drugs on the rate of cutaneous respiration \**

Sub- ject	Drug	CO <sub>2</sub> excreted			O <sub>2</sub> absorbed		
		Control period	After drug	Differ- ence	Control period	After drug	Differ- ence
		cc.	cc.	cc.	cc.	cc.	cc.
9	Epinephrine hydrochloride 1 cc.	140	139	- 1	107	104	- 3
23	Epinephrine hydrochloride, 1 cc.	132	143	+ 11	92	108	+ 16
6	Pituitary extract, 1 cc.	153	149	- 4	113	105	- 8
9	Pituitary extract 1 cc.	154	146	- 8	114	120	+ 6
23	Pituitary extract 1 cc.	156	156	0	103	115	+ 12
6	Histamine hydrochloride, 0.001 gram	151	155	+ 4	113	104	- 9
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6	Pilocarpine hydrochloride 0.008 gram	132	115	- 17	83	83	0
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\* Calculated in cubic centimeters per hour per square meter of skin surface at 27° C

### DISCUSSION

In an earlier investigation (3) repeated measurements were made of the rate of cutaneous respiration under practically identical conditions of temperature and relative humidity in a series of normal subjects. The average individual variation in the rate of carbon dioxide excretion was  $\pm 3$  cc. per hour per square meter of skin surface, and the greatest variation was  $\pm 10$  cc. The average individual variation in the rate of oxygen absorption was  $\pm 5$  cc per hour per square meter of skin surface, and the greatest variation was  $\pm 15$  cc. Since the temperature of the air in the plethysmograph remained practically constant throughout each complete experiment in the present investigation, any change in the rate of carbon dioxide excretion should exceed 20 cc. per hour per square meter of skin surface in order to be significant. Similarly, changes in the rate of oxygen absorption of less than 30 cc. per hour per square meter of skin surface may be disregarded. The rate of cutaneous respiration, therefore, was not significantly affected by any of the drugs employed (Table 1).

The changes produced by the drugs in the color of the skin indicate that cutaneous blood flow had been altered. Although these changes

affect the tension of carbon dioxide and oxygen in the venous blood, their failure to influence the rate of cutaneous respiration suggests that the tension of the gases in that portion of the blood in equilibrium with the tissues had not been altered

Epinephrine, pituitary posterior lobe extract and histamine probably have no significant effect on the metabolic rate of the skin. The accelerated activity of the sweat glands produced by pilocarpine may be accompanied by an increase in the rate of cutaneous metabolism, but the results of the present investigation indicate that this increase, if it occurs, is of very small magnitude in comparison to the total metabolism of the skin

### SUMMARY

1 Measurements were made of the effect of epinephrine, pituitary posterior lobe extract, histamine and pilocarpine on the rate of cutaneous respiration in three normal subjects

2 None of the drugs significantly altered the rate of carbon dioxide elimination and oxygen absorption through the skin

3 The failure to affect the rate of cutaneous respiration indicates that the drugs did not significantly alter the metabolic rate of the skin or the tension of the gases in that portion of the blood in equilibrium with the tissues

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## CUTANEOUS RESPIRATION IN MAN

### VII THE EFFECT OF VENOUS CONGESTION ON THE RATE OF CARBON DIOXIDE ELIMINATION AND OXYGEN ABSORPTION<sup>1</sup>

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This communication presents the results of an investigation of the effect of venous congestion on the rate of cutaneous respiration in man

#### METHODS AND RESULTS

The apparatus employed was that designed by Shaw and his associates (1, 2) The technical procedure was essentially the same as that followed in studying the effect of drugs on the rate of cutaneous respiratory exchange (3) Measurements were made of the total amounts of carbon dioxide eliminated and oxygen absorbed through the skin of the arm in two successive two hour experiments In both periods, the arm was introduced into the plethysmograph to the same extent. The first experiment, in each instance, was a control observation The subject's arm was removed from the plethysmograph between the two periods, and the apparatus was washed out with room air The arm was then reinserted, and a pneumatic cuff, 13 cm wide, was applied After the first gas sample of the second experiment had been collected, the cuff was inflated to a pressure of 40 to 80 mm of mercury, and this pressure was maintained throughout the remainder of the period of observation

The prolonged application of pressure produced extensive edema of the arm It therefore was necessary to measure the surface area and volume of the part within the plethysmograph after both experiments and correct the results of the experiments for the variations observed in these measurements Paresthesia and loss of the sensations of touch and position developed gradually in the hand and wrist during the time the pressure was applied In addition, there was dull aching pain in the arm which became severe toward the end of the observation period On removing the arm from the plethysmograph, transient paresis of the muscles of the hand and forearm usually was observed and, in the experiments with the highest pressures, numerous petechiae were present.

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<sup>1</sup> This investigation was aided by a grant from the DeLamar Mobile Research Fund of Harvard University



Six measurements of the effect of venous congestion on the rate of cutaneous respiration were made in three normal subjects. All observations were made under controlled conditions of temperature and humidity, and the results of each experiment were corrected to the value they would have had if the temperature within the plethysmograph had been 27° C (4). The results of the study are presented in the table.

TABLE I

*The effect of venous congestion on the rate of cutaneous respiration \**

Subject	Pressure applied	CO <sub>2</sub> excreted			O <sub>2</sub> absorbed		
		Control period	During venous congestion	Difference	Control period	During venous congestion	Difference
	mm Hg	cc.	cc.	cc	cc	cc	cc
6	60	155	157	+ 2	83	86	+ 3
6	40	142	133	- 9	96	120	+ 24
6	50	114	157	+ 43	82	94	+ 12
9	50	135	123	- 12	80	86	+ 6
23	80	124	113	- 12	84	90	+ 6
23	60	128	140	+ 12	90	95	+ 5

\* Calculated in cubic centimeters per hour per square meter of skin surface at 27° C

## DISCUSSION

With the method utilized, changes in the rate of oxygen absorption through the skin of less than 30 cc per hour per square meter of skin surface and in the rate of carbon dioxide elimination of less than 20 cc per hour per square meter of skin surface cannot be considered significant (3). The maximum change in the rate of oxygen absorption observed in the present investigation was 24 cc per hour per square meter of skin surface, and in only one instance was the rate of carbon dioxide elimination altered by more than 20 cc per hour per square meter of skin surface. It is concluded, therefore, that venous congestion of the degrees employed does not significantly affect the rate of cutaneous respiration.

The metabolic requirements of the skin are supplied in part by the blood and in part by the exchange of carbon dioxide and oxygen between the air and the tissues. Since it may be assumed that the metabolic rate of the skin remained constant during our experiments, the failure of venous congestion to affect the rate of cutaneous respiration indicates that there had been no significant alteration in the rate of exchange of gases between the blood and the skin. When pressure of 40 to 80 mm of mercury is applied to the upper arm by means of a pneumatic cuff, the flow of blood through the limb is promptly retarded. As a consequence, active compensating dilatation of the minute vessels develops, and the circulation to the extremity returns toward its normal state (5). The

failure of venous congestion to affect the rate of exchange of carbon dioxide and oxygen between the blood and the skin probably is due to this vasodilatation

Our results differ from those of Barratt (6) who observed that the application of a ligature to the upper extremity caused a definite increase in the rate of carbon dioxide elimination through the skin of that arm. Barratt employed higher pressures than we did for he stated that, in his subjects, the radial pulsation was barely perceptible after applying the ligature. In our subjects the pressure in the cuff did not exceed diastolic blood pressure and the volume of the pulse was not appreciably altered. Blood flow through the arm, therefore (7), was decreased to a less extent in our experiments than in those of Barratt.

An extreme reduction in blood flow through the arm would be accompanied by an increased carbon dioxide tension of the capillary blood and a diminished oxygen tension. A rise in carbon dioxide tension of the blood results in an increased rate of carbon dioxide elimination through the skin, and a decrease in oxygen tension causes an accelerated rate of oxygen absorption (8). The observation of Barratt (6) that the rate of carbon dioxide elimination through the skin increased after the application of a ligature to the arm therefore indicates that the ligature had produced an extreme reduction of blood flow through the arm.

### SUMMARY

1 The rates of carbon dioxide elimination and oxygen absorption through the skin of the arm were measured before and during the presence of venous congestion produced by applying pressures of 40 to 80 mm of mercury to the upper arm.

2 Venous congestion did not alter the rate of cutaneous respiratory exchange.

3 The results of the investigation indicate that venous congestion of the degrees employed has no effect on the rate of exchange of carbon dioxide and oxygen between the blood and the skin.

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# STUDIES OF CALCIUM AND PHOSPHORUS METABOLISM

## XIV THE RELATION OF ACID-BASE BALANCE TO PHOSPHATE BALANCE FOLLOWING INGESTION OF PHOSPHATES<sup>1</sup>

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The effect of phosphate-feeding upon the calcium metabolism of man has been reported in experiments previously described in this journal (1). It appeared that over a relatively short period (ten days) the addition of inorganic phosphate to the control diet produced no obvious alteration of the endogenous calcium excretion, fecal or urinary. This was true whether the phosphate administered was acid phosphate (mono-sodium), basic phosphate (di-sodium), or a mixture of these two salts. Such additions to the neutral control diet, however, did produce definite alterations in the total acid-base economy of the body, not previously described, which it is the purpose of this paper to report.

The chemical principles which present themselves as the logical guides to such studies of mineral metabolism have long been recognized, but it is only within recent years that work like that of Gamble, Blackfan, and

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Hamilton (2) has afforded a sound scientific interpretation of the salt action of acid-producing salts. Much emphasis has hitherto been laid upon alterations in the economy of fixed base or upon the fate of the specific ions administered. The comparative rôle of phosphate in this process has received but little scrutiny, however, particularly as reflected in the metabolism of phosphate at various levels of acid elimination. Some authors (3) have suggested that when inorganic phosphate is fed, the metabolic fate of the phosphorus is to some degree predetermined by the potential acidity of the salt fed. Sodium acid phosphate is, indeed, a natural physiological waste product. Does the administration of this acid salt provoke diuresis or loss of fixed base? The companion buffer salt disodium phosphate exists in all body fluids. What is the effect of its administration on bodily economy?

### PLAN OF STUDY

In order to determine the effect of the potential acidity of ingested phosphate upon its assimilation and subsequent fate, we have studied three adult humans whose clinical conditions are described in the addenda. These subjects were placed on a potentially neutral diet, low in phosphorus content, which was continued (essentially) unvaried throughout the experiment. One week was allowed in each case to permit the body to readjust itself to this constant diet before the experiment was begun. After control levels of excretion had been determined, weighed amounts of chemically pure inorganic phosphate were added to the diet, and the resulting change in excretion observed. Three types of inorganic orthophosphate were fed, viz., sodium acid phosphate, disodium phosphate, and an equimolecular mixture of the two. The object was to administer as much phosphate as possible without producing catharsis. Subject RN was given these salts in sequence, so that there are two observations on acid phosphate (subjects WN and RN).

Because of the well recognized lag in excretion following the administration of most medication, the metabolism of phosphorus was followed after the inorganic phosphate was omitted. In all cases, the resumption of control excretion levels indicated that a "steady state" had again been reached in the second period following the last in which phosphate was fed. In this paper, therefore, the acid-base balance was struck as soon as the phosphate balance had approached its former level. It was essential in so doing not to delay too long lest the immediate metabolic response to phosphate feeding be obscured by subsequent readjustments. In evaluating the response of the organism to these phosphate additions, it is assumed that the effect of the control diet on the total metabolism remained constant, and that increases in the potential acidity of the diet (in the form of added phosphate) would be reflected by corresponding alterations in acid excretion.

In these experiments the method suggested by Sherman and Gettler (4) for calculating the potential acidity of food-stuffs was utilized, and the relative amounts of various food stuffs so balanced that the total potential acidity (when oxidized) would be zero. The actual arithmetical operations and sources of error have been illustrated elsewhere (5). The use of such a "control" diet, constantly continued throughout the experiment, obviates any gross errors due to inaccuracy in the composition of the food. Our control diets were low in calcium and phosphorus but adequate with respect to other inorganic salts, vitamins, fat, carbohydrate, protein, and total caloric content.

## EXPERIMENTAL METHODS

The technic followed in these experiments has been given in a description of the special study ward on which our subjects were kept (6). Ammonia and titratable acidity were, as usual, determined within a few hours after mixing of the combined twenty-four-hour urine. The possibility of an important change occurring under these conditions was excluded (7) by comparing the twenty-four-hour analysis with the summated results of successive specimens freshly voided.

Diuresis was excluded by recording nude weight at the same time each day, and showing this to be constant. The time of the experiments was the early (New England) spring months when perspiring was at a minimum. Basal metabolic rates were determined with the Benedict-Roth apparatus.

The analytical methods employed were outlined in our previous publication (1). Through the courtesy of Dr Arlie V. Bock the blood  $\text{CO}_2$  level of subject RN was established (with the apparatus of Neill and Van Slyke) before and at the end of acid phosphate administration. Haldane's negative experiment (8) determining the effect on blood  $\text{CO}_2$  of large doses of basic phosphate obviated the necessity of such determinations in the basic phosphate experiments.

## EXPERIMENTAL RESULTS

Our analysis of the experimental results is based upon the assumption that when an organism exhibits a constant excretion in response to a constantly continued "control" dietary regimen, additions to the diet will be reflected by changes in excretion, the quantitative value of which should be measured from the original excretion level. In presenting results, therefore, emphasis has been laid in this paper upon intakes or outputs *over and above* the corresponding figures for the preliminary "control" (basal) diet. The justification for this procedure will appear in the results cited below.

In presenting these results, furthermore, we have departed from the usual procedure of sharply separating analytical figures from the dis-

cussion of their significance. This method seemed advisable because the paper is in essence a mathematical analysis of metabolic balances and derived equivalents. The massed results of the laboratory determinations may be had (for reference) in our previous paper (1).

*I The influence of the potential acidity of ingesta on inorganic phosphorus metabolism*

Studies on the storage of calcium and phosphorus in rickets have demonstrated that the potential acidity of the diet is an important factor in phosphate metabolism. Zucker, Johnson, and Barnett (3) reported that the change in the acidity of diets from the alkaline toward the acid side might result in healing of the rachitic lesions. Conversely, McClendon (9) reported that adding alkali to a diet increased its power to produce rickets. The fact that most rickets-producing diets are potentially alkaline is of significance.

Most illuminating from this point of view are experiments which determine the fate of phosphate of known potential acidity added to a consistently continued basal or control diet. Karelitz and Shohl (10), in studying the metabolism of phosphate in rachitic rats to whose diet (a potentially alkaline one) acid phosphate was added, found that this addition resulted in a reversed excretion ratio between urine and feces. In the period preceding the feeding of phosphate only 7 per cent of the excretion was urinary, whereas with increase of phosphate in the diet the urinary moiety was about half, being increased both relatively and in an absolute sense. Despite the fact that their animals were in a state of phosphorus starvation, only one-fourth of the phosphate eaten was retained on this diet (which was potentially alkaline, even though acid phosphate had been added). The possibility that the greater excretion of phosphate in the urine under a more acid regimen might be due to greater absorption from the intestines was investigated in man by Zucker (11), who added NaOH and HCl to the control diet, which, however, was evidently potentially acidic as indicated by a titratable acidity of over 700 cc N/10 acid daily. The greater absorption and retention of phosphorus under the influence of acid was also confirmed by Scheer (12).

From the massed analytical results previously published (1), the phosphate determinations have been abstracted and combined in Table I to indicate the respective phosphorus balances for the three types of salt fed, i.e., acid, basic, and the equimolecular mixture of the two. Stress is laid upon the values headed "balance above control," because these derived values indicate the extent of fluctuation above the basal level. In italics (beside the excretion figures for each three-day period) is given the relative participation (expressed as percentage) of urine and feces, respectively, in the combined phosphate excretion for that period.

TABLE I  
Excretion of phosphate in urine and feces following ingestion of inorganic phosphate

Subject	Three-day period	Phosphorus balance				Phosphorus balance above control						Phosphorus retained above control	Calculated acid equivalent of retained phosphorus	cc. N/10	
		Urine	Feces	Total output	Total intake	Urine*		Feces*		Total* output	Total* intake				
						grams	per cent	grams	per cent						
WN Acid phosphate alone	(I)	1.1	(0.5)	(1.7)	(1.9)										
	(II)	(1.3)	(0.6)	(1.8)	(1.9)										
	III†	1.3	0.6	2.0	1.9										
	IV†	2.2	0.6	2.8	3.9	0.8		- 0		0.8	2.0	1.2	316		
	V†	7.3	1.6	9.0	10.8	6.0	86	1.0	14	7.0	8.9	2.0	530		
	VI†	6.8	2.1	8.8	10.8	5.4	79	1.4	21	6.8	8.9	2.1	564		
					Total	12.2	83	2.4	17	14.6	19.8	5.3	1410		
	VII	1.6	0.9	2.5	1.3	0.2		0.3		0.5	-0.6				



cussion of their significance. This method seemed advisable because the paper is in essence a mathematical analysis of metabolic balances and derived equivalents. The massed results of the laboratory determinations may be had (for reference) in our previous paper (1).

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Excretion of phosphates in urine and feces following ingestion of inorganic phosphate

Subject	Three-day period	Phosphorus balance				Phosphorus balance above control						Phosphorus retained above control	Calculated acid equivalent of retained phosphorus	cc. N/10
		Urine	Feces	Total output	Total intake	Urine*		Feces*		Total* output	Total* intake			
						grams	per cent	grams	per cent					
WN Acid phosphate alone	(I)	2.2	0.6	2.8	3.9	0.8		- 0		0.8	2.0	1.2	316	
	(II)	7.3	1.6	9.0	10.8	6.0	86	1.0	14	7.0	8.9	2.0	530	
	III†	6.8	2.1	8.8	10.8	5.4	79	1.4	24	6.8	8.9	2.1	564	
					Total	12.2	83	2.4	17	14.6	19.8	5.3	1410	
	IV‡													
	V‡													
	VI‡													
	VII‡													
	VII	1.6	0.9	2.5	1.3	0.2		0.3	0.5	-0.6				

TABLE I (continued)

Subject	Three day period	Phosphorus balance				Phosphorus balance above control						Phos- phorus retained above control	Calculated† acid equivalent of retained phosphorus	
		Urine	Feces	Total output	Total intake	Urine*		Feces*		Total* output	Total* intake			
						grams	per cent	grams	per cent					
RN Acid phosphate alone	X†	48	26	74	90	38	67	19	33	57	71	14	385	
	XI†	54	13	68	89	44	86	07	14	50	71	21	562	
	XII	25	08	33	18	15	94	01	6	16		-16	-419	
					Total	97	78	27	22	123	142	19	528	
	Average¶	12	07½	18	16									
Basic phosphate alone	(I)	(14)	(05)	(19)	(18)									
	II¶	13	07	20	18									
	III¶	13	06	19	18									
	Average¶	13	06	20	18									
	V†	32	33	65	75	18	40	27	60	45	56	11	-61	
	VI†	53	19	73	87	40	75	13	24	53	70	16	-85	
	VII†	50	13	63	84	37	86	06	14	43	70	23	-124	
	VIII	16	08		Total	95	67	46	33	141	196	50	-270	
				24	16									

TABLE 1 (continued)

Subject	Three-day period	Phosphorus balance				Phosphorus balance above control				Phosphorus retained above control	Calculated† acid equivalent of retained phosphorus
		Urine	Feces	Total output	Total intake	Urine*	Feces*	Total* output	Total* intake		
LZ Equimolecular mixture of basic and acid phosphate	(I)	gms (1.8)	gms (0.6)	gms (2.4)	gms (2.1)	gms	gms	gms	gms	gms	cc. N/10
	(II)	2.0	1.1	3.1	2.1						
	XI‡										
	XIII‡	3.3	0.7	4.0	5.4	17	— 0	16	3.3	17	
	XIV‡	3.6	1.7	5.2	5.4	19	10	29	3.3	0.4	
		3.3	1.4	4.7	5.4	16	0.7	2.4	3.3	1.0	
					Total	52	17	69	9.9	3.0	
	XV‡	1.6	0.5	2.1	2.1						
	XVI‡	1.3	0.4	1.8	2.1						
	Average of 3 controls‡	1.6	0.7	2.3	2.1						

Data in parentheses not used because of the possibility of metabolic flux before "steady state" is attained.

\* Derived from the corresponding columns under phosphorus balance, preceding

† Derived from the preceding column.

‡ Phosphate fed during these periods

§ Actual analysis lost Assume figure of period XIV

|| Corrected for slight variations in diet

¶ Taken as control

The corrected phosphorus balance appears in the next column (next to the last) The final column indicates the acid equivalent of the phosphorus retained, calculated with reference to blood pH = 7.4, alkali retention is indicated (in the basic phosphate experiment, subject RN) by minus signs

The first two experiments (subjects WN and RN) are devoted to sodium acid phosphate, the third to disodium phosphate, and the fourth to an equimolecular mixture of the two

*When inorganic phosphate is fed, does the potential acidity of the salt fed influence the phosphorus balance?*

In sodium acid phosphate experiment WN, 5.3 grams out of the 19.8 grams of phosphorus fed were presumably retained, i.e., 27 per cent In sodium acid phosphate experiment RN, 1.9 grams out of the 14.2 grams of phosphorus fed were presumably retained, i.e., 13 per cent The average of the two experiments is 20 per cent (roughly one-fifth) presumably retained

In disodium phosphate experiment RN, 5.0 grams out of the 19.6 grams of phosphorus fed were presumably retained, i.e., 25 per cent (roughly one-fourth)

In the equimolecular phosphate experiment LZ, 3.0 grams out of the 9.9 grams of phosphorus fed were presumably retained, i.e., 30 per cent (roughly one-third)

One might summarize these various results by stating that irrespective of the potential acidity of the inorganic phosphate fed, one-fourth of the phosphate fed is presumably retained in these relatively short observations.

*When inorganic phosphorus is fed, what is the partition of phosphate excreted in urine and feces?*

It will be observed that considerable fluctuations occurred from period to period in the division of phosphate between urine and feces It will be more profitable, therefore, to consider the total excretion for each experiment

In sodium acid phosphate experiment WN, 2.4 grams out of the 14.6 grams of phosphorus recovered appeared in the feces, i.e., 17 per cent, leaving 83 per cent as urinary In the duplicate experiment on subject RN, 2.7 grams out of the 12.3 grams of phosphorus recovered appeared in the feces, i.e., 22 per cent, leaving 78 per cent as urinary The average values for sodium acid phosphate excretion, therefore, are fecal, 19 per cent (roughly one-fifth), and urinary, 81 per cent (roughly four-fifths)

In disodium phosphate experiment RN, 4.6 grams out of the 14.1 grams of phosphorus recovered appeared in the feces, i.e., 33 per cent (roughly one-third), leaving 67 per cent (roughly two-thirds) as urinary

In the equimolecular mixture experiment LZ, 1.7 grams out of the 6.9 grams recovered appeared in the feces, i.e., 25 per cent (roughly one-fourth) leaving 75 per cent (roughly three-fourths) as urinary

One might summarize these results by stating that irrespective of the potential acidity of the extra inorganic phosphate fed, one-fourth of the excreted phosphorus is fecal. During the control periods, on a potentially neutral mixed diet, one-third of the total phosphorus excretion was fecal.

The net result of the phosphorus balance presented in Table I is (first) that irrespective of the potential acidity of the salt fed, one-fourth of the phosphorus is retained, and (secondly) that irrespective of the potential acidity of the salt fed, one-fourth of the excreted phosphorus is fecal.

## *II The influence of the potential acidity of ingesta upon total fixed base metabolism*

The relation of phosphate excretion to the excretion of total acid, especially from the viewpoint of its bearing on urinary acidity, has occupied the interests of many investigators of acid-base economy. Polin (13) in 1903, in discussing the acidity of urine, pointed out the convenience of the assumption that the titratable acidity of urine is due to acid phosphate. The classical work of Henderson (14) on the excretion of acid catabolites emphasized the importance of using the hydron concentration of the blood as a point of reference in titrating acid excretion, and clarified the significance of urinary hydron concentration in terms of the titration curve of orthophosphoric acid. The chemical mechanisms by which phosphate is metabolized, however, demand further clarification.

### *The neutralization of phosphate within the bowel*

When an inorganic salt or acid is fed, fixed base is added or withdrawn by the intestinal juices so that the potential acidity of the original salt is altered. If the potential acidity be far removed from neutrality, this process may be spoken of as "neutralization" of the salt by the bowel. The analogy is admittedly crude: first, because the pH to which the salt is neutralized is not known exactly, and secondly, because complex organic buffer-substances within the gut make exact physicochemical reasoning impossible. Nevertheless, such a concept may be profitably applied to the fate of ingested phosphate when analyses are made on aqueous extracts of fecal ash by assuming that "neutralization" occurs with reference to blood pH = 7.4, or intestinal pH = 8. It is not easy to decide which of these hydrogen ion values is the preferable reference point. Fortunately, however, the base bound by phosphate between these two limits is relatively small and either point may, therefore, be used for practical purposes.

The original potential acidity of the phosphorus found (in feces) can be calculated as its base equivalent, i e , 1.8 times the mols of phosphorus involved. When fecal fixed base is determined, the extent of "neutralization" of the unabsorbed inorganic phosphate may be estimated. From the massed analytical data previously published (1), the values for fecal phosphorus and fecal fixed base have been abstracted, and are presented in Table II for comparison. Because of fluctuations from period to period only totals are discussed.

In sodium acid phosphate experiment WN, the extra fixed base (967 cc N/10) more than neutralized the potential acidity (*b*) of the phosphate present (637 cc N/10, referred to pH = 7.4). The extra base (*c*) is in fact greater than the molal equivalent (*a*) of the extra phosphorus present (768 cc M/10). In sodium acid phosphate experiment RN, the extra fixed base (897 cc N/10) more than neutralized the potential acidity (*b*) of the phosphate present (718 cc N/10, referred to pH = 7.4). The extra base in this instance is approximately equal to the molal equivalent of the extra phosphorus present (865 cc M/10).

These results may be explained on the basis that the acid phosphate which escaped absorption (or was re-excreted) was excreted chiefly in the form of disodium phosphate. The figures, indeed, indicate for WN a fraction excreted as tertiary phosphate, and for RN a small fraction excreted as primary phosphate. Such differences, however, are probably not significant in a metabolic procedure of this sort.

In the disodium phosphate experiment RN, the extra fecal phosphorus (*a* = 994 cc M/10) would theoretically have carried with it, if excreted unchanged, 1988 cc N/10 fixed base (i e , sodium). The extra fixed base (*c*) actually found (2167 cc N/10) is in fair agreement, and indicates that this phosphate was excreted chiefly in the form in which it was fed.

The conclusion indicated by these three experiments is that fecal inorganic phosphate was excreted chiefly as the disodium salt irrespective of the form in which it was fed. It is interesting that Berg (15) believed calcium phosphate to be excreted by bowel in tribasic form. It is clear, however, that such a condition cannot be ascribed to the total fixed base of the feces when phosphate is fed. The fact that calcium is a weaker base than sodium may explain this apparent discrepancy.

#### *The neutralization of phosphate in the urine*

In the preceding section, the base equivalent of the fecal phosphate excreted was found to serve as a rough measure of the extra fixed base excreted (by bowel) when inorganic phosphate was fed. In the case of urinary excretion, an even more precise relationship exists—at least for acid phosphate. For the sake of clearness and brevity the analytical values for individual successive periods have been omitted from Tables III and IV, and only the summated values (representing nine consecutive

TABLE II  
*Faecal base following inorganic phosphate feeding*

Subject	Three-day period	(a)		Calculated acid equivalent of faecal phosphorus†	(b)	Calculated sodium due to extra phosphorus above control	Actual faecal fixed base	(c)
		cc M/10	cc M/10					
WN Sodium acid phosphate	III§	205	cc. N/10	170	cc. N/10	cc. N/10	cc. N/10	cc. N/10
	IV†	200	— 5	166	— 4		836	— 134
	V†	522	317	433	263		1431	461
	VI†	661	456	548	378		1610	640
			Total 768		Total 637			Total 967
RN Sodium acid phosphate	X†	836	623	694	517		1740	1000
	XI†	426	213	354	177		775	35
	XII	242	29	201	24		602	— 138
			Total 865		Total 718			Total 897
	XIV§	213		177			740	



remains undisturbed by the addition of phosphate The results make it likely that the premise is justified

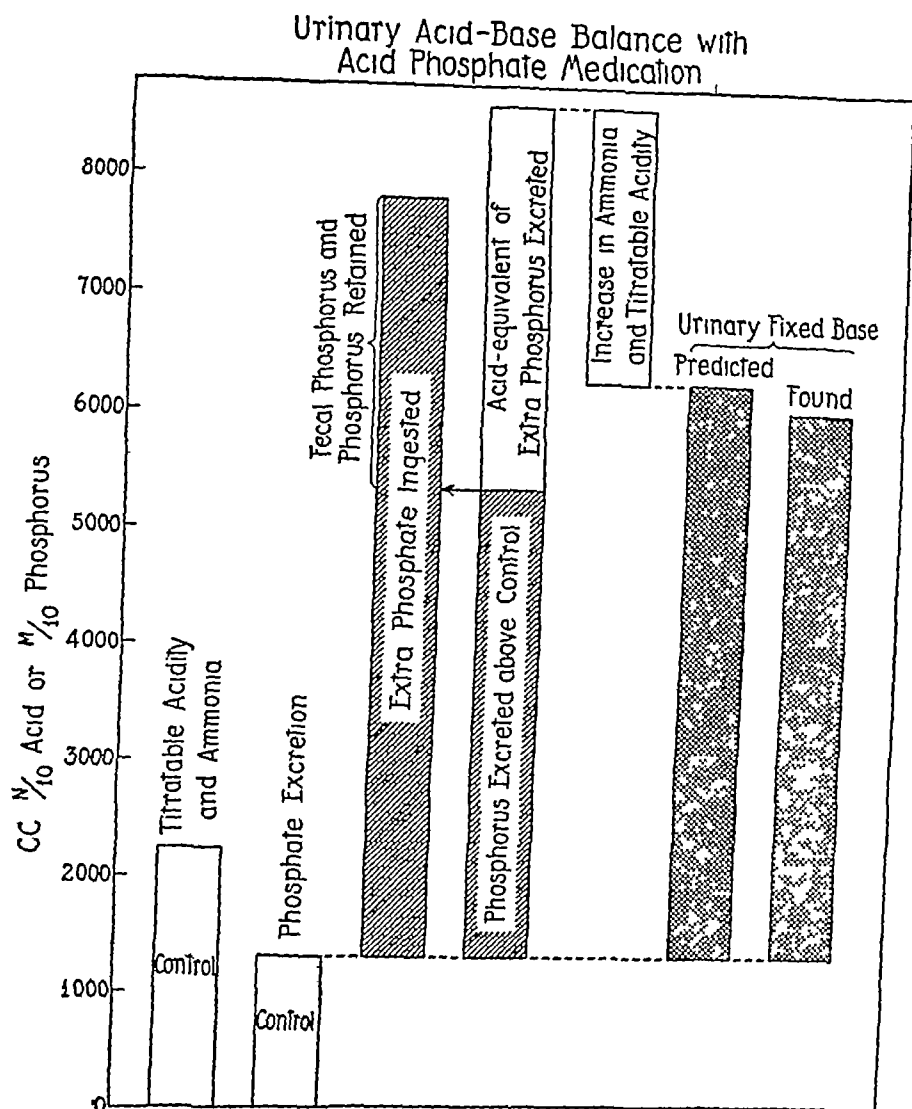


FIG 1 PATIENT WN—THE ACTUAL INCREASE IN URINARY FIXED BASE IS APPROXIMATELY EQUIVALENT TO THAT THEORETICALLY PREDICTED

The control excretions are pictured at the left of the chart The superimposed phosphorus excretion is indicated by the hatched areas

#### *Basic phosphate in urine*

The foregoing prediction of fixed base excretion depended upon the assumption that the underlying general trend of inorganic salt metabolism remained undisturbed by the feeding of acid phosphate When given a constant control diet, each subject had eventually arrived at a plateau level of excretion for the several dietary constituents measured The

TABLE IIIA<sup>a</sup>

### Urinary acid balance due to acid phosphate fed

[illegible]

TABLE IIIA (continued)

Subject	Three day period	Urinary phosphorus	Urinary phosphorus above control			Neutralization of extra acid above control			Total extra base predicted excreted	Analyses of actual total fixed base of urine	Actual extra fixed base†
					Acid equivalent = 0.83 X molal equivalent‡	By extra ammonia§	By extra titratable acidity§	Remaining extra acid Neutralized by base supplied by body			
RN	X XI XII	grams 4.83 5.44 2.54	gramst 3.77 4.38 1.48	cc M/10† 1220 1410 480	cc N/10 1014 1178 396	cc N/10 72 -52 -40	cc N/10 616 486 128	cc N/10 326 744 308	cc N/10 1546 2154 788	cc N/10 3570 4740 4260	cc N/10 810 1980 1500
	(I) XIII   XIV   Average	(1.4) 1.15 0.97 1.06	Totals	3110	2588	-20	1230	1378	4488		4290
										2840 2680 2760	

\* This table gives in full the data summarized in Table III

† Derived from the column immediately preceding

‡ Referred to the pH value of the blood (= 7.4)

§ Data taken from Table 2, first two columns, published in this Journal, Vol. X, 256

|| Taken as control

various factors which had determined the height of the plateau level are, of course, no better understood than are the factors which determined the phosphorus balance itself. The addition of acid phosphate to the control diet, merely produced an incremental change in the excretion plateau values which was assumed to be additive.

This assumption could not be maintained, however, when basic phosphate was fed. Table IV indicates that a fundamental shift in the

TABLE IV

(Patient RN)—Phosphate balance and acid-base balance following the ingestion of disodium phosphate

	Intake	Output			Balance	Remarks
		Urine. Change from control level	Feces. Change from control level	Total. Change from control level		
Phosphorus grams	19.6	9.5	4.6	14.2	(4.9)*	
cc. N/10 anion	11560	5600	2712	8320	(2890)*	
Per cent	100	49	24	72	28	
Titrateable acid cc. N/10	-1090	-1732			642	Gamble's base economy More alkalinity appears in the urine than was fed
Ammonia cc. N/10		-1041				
Titrateable acid plus am- monia cc. N/10		-2773				Henderson's total acid the best measure of saving of fixed base apparently indicates much less saving of fixed base
Fixed base cc. N/10 Per cent	12670 100	2565 20	2167 17	4732 37	7938 63	A large positive fixed base balance
Total anion (calculated)		-208				Gamble's 'total acid' drops even though there are 5600 milliequivalents additional HPO <sub>4</sub> present

\* This figure has been modified by a slight correction for refused food

electrolytes of the body occurred when the alkaline salt was administered. In short, the fundamental excretion plateau was altered. That this was so is suggested by several facts shown in the table. More titrateable alkalinity appeared in the urine than the alkali equivalent of the salt fed. Henderson's "total acid" (14), which is the best measure of saving of

fixed base, indicated less base economy than before, but on the contrary the actual analyses showed a large positive fixed base balance. Moreover, Gamble's "total acid" (17) (i.e., total anion) dropped from its control level, despite the fact that much extra phosphate ion was present.

This last fact makes it seem likely that the extra phosphate was excreted in preference to other anions (e.g., chloride or carbonate). Unfortunately, it was not possible in this study to have a complete analytical synopsis of anion excretion. Such observations would of necessity entail analyses for the high carbonate content of alkaline urines (described by Gamble (18)). Our findings, inadequate as they are, are reported here to show the need of more detailed study, which the phenomenon merits in view of its bearing upon the effect of alkaline diuretics.

### DISCUSSION

This investigation was undertaken with the supposition that phosphate and acid exerted reciprocal effects upon their mutual and respective metabolisms. The object of this study was to measure the extent of this mutual influence when inorganic phosphate was fed to adult humans over a relatively short period.

The first question to be answered was: How does the potential acidity of the inorganic phosphate fed affect the assimilation of phosphorus? The answer given by these experiments is essentially negative. No definite influence of potential acidity could be discerned upon the absorption or retention of inorganic phosphorus.

The second question to be answered was: How does the potential acidity of the inorganic phosphate fed affect the fixed base metabolism while the extra phosphate is being excreted? In analyzing this problem, two rational mechanisms were encountered which explained quantitatively the excretion of total fixed base (i.e., cation considered in its rôle as an electrolyte, apart from its alkaline properties). The first of these assumed that fecal phosphate was excreted chiefly as disodium phosphate, regardless of its potential acidity when fed. The second of these assumed that when the kidney failed to neutralize acid phosphate completely (either with ammonia or by altering titratable acidity), the deficiency was made up quantitatively by draught upon the body's reserve stores of fixed base.

It is of passing interest that the subjects here described apparently retained about one-fourth of the phosphate fed (irrespective of the potential acidity of the salt administered). Of more moment is the discrepancy between the respective amounts of acid (or base) recovered and the corresponding portions of phosphate recovered. In the acid phosphate experiments four-fifths of the extra phosphate was found in excreta, in which only one-third of the corresponding acid could be measured. In the basic phosphate experiment, three-fourths of the

administered phosphate was recovered, but only about one-third of the fixed base.

### CONCLUSIONS

The influence of the ingestion of sodium acid phosphate and disodium phosphate on the balance of inorganic electrolyte was studied in subjects on a constant, potentially neutral diet

1 About one-fourth of the phosphate fed was stored Approximately one-fourth of the excess phosphate excretion was fecal

2 Fecal inorganic phosphate was excreted chiefly as the dibasic salt irrespective of the form in which it was fed

3 With acid phosphate there was an increased excretion of inorganic base which was mainly urinary It was equivalent to the theoretical amount of base necessary to neutralize the excess phosphoric acid excretion, not conserved by titratable acidity and ammonia

4 This simple mechanism, however, did not hold when disodium phosphate was administered The mechanisms here involved are not yet obvious

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#### DESCRIPTION OF PATIENTS

A brief description of each of the patients used in this investigation follows

*WN* A married female of 34 years of age, weighing 89 kilos, suffering from chronic atrophic arthritis of 2 years' duration (M G H number 289324)

*RN* A single female of 39 years of age, weighing 56 kilos, suffering from rheumatic heart disease (mitral stenosis), chronic bronchitis, and bronchial asthma During the period of observation she had no cough, her chest was clear, and there were no signs of myocardial failure (M G H number 286369)

*LZ* A male, aged 18, weighing 60 kilos, recovering from chronic multiple neuritis, confined to bed because of weakness of extremities, but feeling well (M G H number 274029)

## STUDIES IN PARATHYROID PHYSIOLOGY

### III THE EFFECT OF PHOSPHATE INGESTION IN CLINICAL HYPERPARATHYROIDISM<sup>1</sup>

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In the second paper of this series (1) the disorders of calcium phosphate metabolism were divided into three fundamental groups. It was believed that under normal conditions body fluids contain all the calcium phosphate which that particular fluid system can hold at that particular time. The first and commonest group was that in which the body fluids contain less than this saturating amount of calcium phosphate. Because of this, calcium phosphate is not deposited into osteoid tissue with the resulting pathological picture of wide osteoid seams on the trabeculae (cf. rickets and osteomalacia). The second group was that in which the body fluids, because of certain extraordinary circumstances, contain more than the normal quota of calcium phosphate and deposition in tissues other than osteoid tissue results (e.g. ergosterol poisoning and cases of metastatic malignancy and myeloma). Finally, a third possibility presented itself. It is conceivable that the body fluids might contain a normal saturating amount of calcium phosphate, but that the proportion of the calcium ions to the phosphate ions might be abnormal. Such, it was believed, is the disorder in diseases of the parathyroid glands. To recapitulate then, we conceived of three possible variations from the normal saturation of body fluids with calcium phosphate: (a) subsaturation, (b) supersaturation, and (c) anomalous-saturation (i.e. quantitatively normally but qualitatively abnormally saturated).

In the first paper of this series (2), the hypothesis was advanced that this abnormality in the relation of the calcium ion to the phosphate ion in parathyroid disorders was dependent upon changes in phosphorus excretion in the urine brought about by the parathyroid hormone. Thus it was believed that on administration of the parathyroid hormone the first effect was an increased phosphorus excretion, that this resulted in a decreased serum inorganic phosphorus, that this tended to leave the serum's capacity to take up calcium phosphate unfulfilled; that calcium

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<sup>1</sup> Delivered in abstract at the meeting of the American Society for Clinical Investigation, May 4, 1931, Atlantic City, N. J.



phosphate was mobilized from the bones to meet this tendency to a deficiency, that equilibrium was finally established with the blood phosphorus slightly lowered and the blood calcium slightly raised, and that, as a result of the raised blood calcium, the urinary calcium excretion was increased. No hypothesis was offered to explain the mechanism of this initial increased phosphorus excretion. The known facts would be explained if we hypothesized a sudden lowering of the threshold for phosphorus excretion in the urine on the administration of the parathyroid hormone. The cause for this apparent change in the kidney threshold for phosphorus might be clear if we knew what the cause for any kidney threshold was, and will probably not be found in the kidney but rather in some physical-chemical equilibrium in the body as a whole.

If one believes in this hypothesis of Albright and Ellsworth (2), which makes the calcium changes in parathyroid dysfunctions secondary to phosphate changes, it is of course logical to attempt to correct the calcium abnormalities in hyperparathyroidism by first attacking the phosphorus abnormalities. In their experiment III, Albright and Ellsworth (2) were able to influence only slightly the high blood phosphorus of a patient with hypoparathyroidism by first giving a high phosphorus diet and then abruptly changing to a low phosphorus diet. The present investigation is the exact antithesis to the above, it was undertaken to determine whether the low blood phosphorus in hyperparathyroidism can be altered by diet. Bauer, Albright, and Aub (3) pointed out that in clinical hyperparathyroidism "a high phosphorus diet might be more efficacious from a therapeutic standpoint than a high calcium diet."

Whereas it is our working hypothesis that the state of hyperparathyroidism with its high blood calcium and low blood phosphorus is reached because the parathyroid hormone lowers the threshold for phosphorus excretion in the urine, it must be emphasized that this state leads to skeletal decalcification and dephosphatization because of the increased calcinuria resulting from the high blood calcium. Because the blood calcium is not much increased by a high calcium diet, this increased calcinuria, dependent on the height of the blood calcium, is not appreciably increased by a high calcium diet, and the patient with clinical hyperparathyroidism studied by Bauer, Albright and Aub (3) was kept in calcium equilibrium by this means. Thus a high calcium diet merely combats a complication (increased calcinuria) of the state of hyperparathyroidism but does not alter the state. It was to be hoped that a high phosphorus diet would raise the blood phosphorus, as a result lower the blood calcium, and thus decrease the calcium excretion. Thus calcium balance might be obtained likewise, and furthermore, the state of hyperparathyroidism might be altered in the direction of normal.

## EXPERIMENTAL

Three patients with conditions which metabolically correspond to hyperparathyroidism were studied in the Metabolism Ward for fore periods on weighed diets and then under exactly the same conditions except for the addition of large amounts of phosphate to the diet. The urine and feces were analyzed in three-day periods for calcium and phosphorus. The methods for the preparation of the diet and the collection of the excreta were those previously reported (4). Serum inorganic phosphorus and calcium determinations were done on venous blood obtained before breakfast. All calcium determinations were done by the Fiske method (5), and all phosphorus determinations by the Fiske and Subbarow method (6).

*Experiment I*

The subject of this investigation was the same sea captain on whom in 1926 the clinical diagnosis of hyperparathyroidism was made for the first time in this country by Dr Eugene F DuBois. It is not necessary to repeat the details of his history or previous metabolic findings as they have been reported elsewhere (7) (3) (8). The essential features are that he presented all the criteria of hyperparathyroidism,—osteitis fibrosa cystica generalisata, high blood calcium, low blood phosphorus, and increase of calcium and phosphorus in the urine—that at operation, no parathyroid tumor was found, that the situation was little if at all altered by the removal of two small parathyroid glands—that it was found that the decalcification could be combatted by a very high calcium diet, that as a result of a prolonged regime on a high calcium diet the patient was brought from a bedridden state to an economically self-supporting state, but that in spite of all he continued to present all the criteria of hyperparathyroidism.

The patient was studied for three three-day periods on a high calcium, moderately high phosphorus diet. He was then studied for one two-day period and for three three-day periods on the same regime plus large amounts of phosphate by mouth. Periods IV and V, the first two periods on the high phosphate diet, are of little value because of nausea and vomiting occasioned by the ingestion of secondary sodium phosphate. As a result acid sodium phosphate was resorted to. The more neutral salt had been chosen because of the known action of acid salts in increasing the excretion of calcium in the urine. Any diminution in the calcium excretion obtained by the ingestion of the primary sodium phosphate salt may be considered to have occurred in spite of this factor. The  $\text{CO}_2$  combining power of the serum as a matter of fact was little altered, being 47 and 44 volumes per cent during the last period at the height of the acid ingestion. The data are presented in Table I and Chart I.

TABLE I  
*Calcium and phosphorus data of experiment I*

Period	Calcium (per 3 day period)					Phosphorus (per 3-day period)					Serum			Remarks
	Urine	Feces	Total excretion	Intake	Balance	Urine	Feces	Total excretion	Intake	Balance	Ca	P	Ca × P	
	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	mgm per 100 cc	mgm per 100 cc		
I	1 00	1 68	2 68	2 79	+0 11	2 52	0 36	2 88	1 98	-0 90	14 1(1)†	2 80	40	Control period
II	1 51	2 06	3 57	2 79	-0 78	2 83	0 49	3 32	1 98	-1 34	14 6(1)	2 84	41	Control period
III	1 42	2 61	4 03	2 79	-1 24	2 30	0 67	2 97	1 98	-0 99	13 9(1)	2 50	35	Control period
IV*	1 32	2 19	3 51	†		3 63	0 95	4 58	†		14 5(1)	2 62	38	Phosphate ingestion
V	0 88	2 22	3 10	†		3 47	1 07	4 54	†		13 2(2)	3 10	41	Phosphate ingestion
VI	0 73	1 36	2 09	2 79	+0 70	5 25	1 97	7 22	12 18	+4 96	12 2(2)	4 37	53	Phosphate ingestion
VII	0 60	2 06	2 66	2 79	+0 13	6 90	2 30	9 20	12 18	+2 98	12 8(1) 12 9(2) 12 6(3)	5 90 4 37 5 87	76 56 79	Phosphate ingestion

\* As period IV was a two- instead of a three-day period, values for this period in the chart have been multiplied by 3/2 in order to be comparable

† The intakes in periods IV and V, though high in phosphorus, are not known because of nausea and vomiting occasioned by the secondary sodium phosphate ingestion (see text)

‡ The arabic numeral in the parenthesis indicates on which day of the period, first, second, or third, the blood specimen was collected

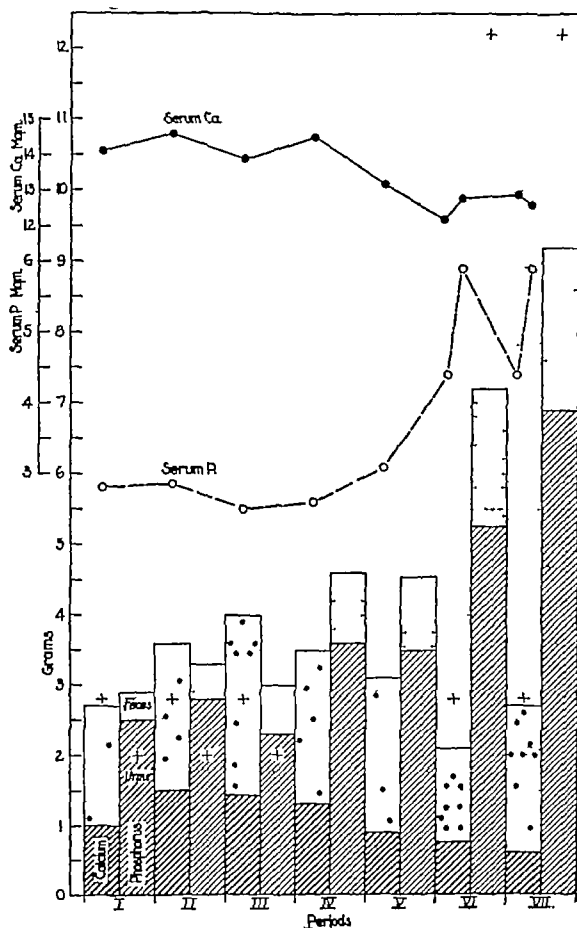


CHART I CALCIUM AND PHOSPHORUS DATA FOR EXPERIMENT ONE

Heavily hatched and dotted columns indicate Ca excretion, lightly hatched and dotted columns indicate phosphorus excretion. Hatched area urinary excretion, dotted area fecal excretion. + indicates intake.

During the three control periods the expected findings were present. The serum calcium was very high (14.1 mgm per 100 cc), the serum phosphorus was correspondingly low (2.8 mgm per 100 cc), the calcium in the urine was markedly elevated (1.00 gram in 3 days), and the ratio of phosphorus in the urine to phosphorus in the feces was very high. The serum phosphorus was slightly higher than in 1926 when it averaged about 2.0 mgm per 100 cc. The calcium excretion in the feces was much higher than one would have expected from the 1926 studies. At that time on a calcium intake of 3.22 grams per three-day period, the fecal calcium was 1.68 gram. The high calcium intake in the present investigation was obtained by adding calcium gluconate to the diet whereas in 1926 it was obtained by altering the diet itself.

Examining first the phosphorus changes, one notes that with the establishment of the high phosphorus intake most of the phosphorus was absorbed as judged from the fecal values, but that there was an immediate and marked increased excretion of phosphorus in the urine. The increased urinary phosphorus excretion did not, however, compensate for the increased phosphorus absorption and the serum phosphorus rose (2.5 mgm to 5.9 mgm). Judging from the rapidity with which the urinary phosphorus excretion rose one can infer, we believe, that the low serum phosphorus at the outset was not below the threshold for phosphorus excretion, but that the threshold itself was low. Comparing period VII with period I, one notes that about 4.0 grams more phosphorus were excreted in the urine in period VII than in period I. This means that 4.0 more grams of phosphorus went from the blood into the urine. However, if one assumes that the blood volume was 5 liters for rough calculations, there was only about 150 mgm increase in phosphorus content in the entire blood in period VII over period I. It would appear that the kidney was making every effort to excrete the added absorbed phosphorus and was only about 150 mgm behind at the end of the investigation. One gets the impression that any changes brought about by the high phosphorus ingestion are merely due to the slight lag in the ability of the kidney to establish equilibrium.

Turning to the calcium data one notes that as the serum phosphorus rose the serum calcium fell. However, one notes that the  $\text{Ca} \times \text{P}$  product rose very appreciatively. This is in striking contrast to the situation which one obtains when the calcium is lowered by decreasing the degree of hyperparathyroidism (1). This again suggests that the increased absorption of phosphorus was too rapid to allow equilibrium to be established. As was to be expected, with the lowering of the blood calcium there was a corresponding lowering of the urinary calcium excretion. The fecal calcium excretion was not affected. It would appear that fecal calcium excretion is not a threshold phenomenon (cf normal fecal calcium excretion in hypoparathyroidism at a time when the

serum calcium is below the threshold for urinary calcium excretion) With the increase in the  $\text{Ca} \times \text{P}$  products it is probable that the tendency to deposit calcium phosphate into the bones was increased and this is borne out by the tendency for the balances of Ca and P to become positive.

The conclusions suggested from experiment I are

*I In clinical hyperparathyroidism the ingestion of large amounts of phosphate results in almost complete absorption of the phosphate into the blood stream*

*II The absorbed phosphates are rapidly excreted by the kidney suggesting that the depressed serum phosphorus level in hyperparathyroidism is not below the threshold for phosphorus excretion, but that the threshold itself is low*

*III In spite of the effort of the kidney to excrete the phosphate, the serum phosphate can be made to rise and the serum calcium to fall by ingestion of huge amounts of phosphate*

*IV However, the fall in calcium does not correspond to the rise in phosphorus and the  $\text{Ca} \times \text{P}$  product rises This rise promotes calcium deposition*

*V Finally, with the lowering of the serum calcium, the urinary calcium excretion is diminished*

*VI The net result of ingestion of phosphate in hyperparathyroidism is the tendency to produce a positive balance of calcium phosphate and the altering of the serum calcium and phosphorus values in the direction of normal*

### *Experiment II*

The subject of this investigation is suffering from a malady which in every respect resembles that of the previous patient and which has run the same course The history and x-ray findings are given in the appendix (q v) The essential facts for the present discussion are that she is a married woman of 41 who entered this hospital with marked osteitis fibrosa cystica generalisata and metabolic evidence of hyperparathyroidism, and that, following the investigation to be reported here, she was operated upon without the finding of a parathyroid tumor, but with the removal of two normal parathyroid glands The operation was apparently without benefit.

The patient was studied for two control periods on a low calcium and normal phosphorus diet. In period III large amounts of tertiary sodium phosphate by mouth were added to the diet This immediately caused nausea and vomiting so that again acid sodium phosphate was resorted to This was well tolerated No marked acidosis was occasioned by this therapy as the  $\text{CO}_2$  combining power of the serum at the end of the investigation was 59 volumes per cent. The data are shown in Table II and Chart II

TABLE II  
*Calcium and phosphorus data of experiment II*

Calcium and phosphorus data of experiment 11

Period	Calcium (per 3-day period)					Phosphorus (per 3 day period)					Serum			Remarks
	Urine	Feces	Total excretion	Intake	Balance	Urine	Feces	Total excretion	Intake	Balance	Ca	P	Ca × P	
	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	mgm per 100 cc.	mgm per 100 cc.		
I	0 71	0 16	0 87	0 22	-0 65	1 09	0 20	1 29	1 67	+0 38	14 2(1)†	2 3	33	Control period
II	0 54	0 20	0 74	0 22	-0 52	1 33	0 44	1 77	1 63	-0 14	14 0(1)	2 4	34	Control period
III	0 59	0 11	0 70	*		4 06	0 32	4 38	*		14 3(1)	2 3	33	Phosphate ingestion
IV	0 26	0 10	0 36	0 22	-0 14	6 74	0 35	7 09	8 51	+1 42	12 3(1)	3 2	39	Phosphate ingestion
											12 2(2)	3 1	38	
V	0 31	0 11	0 42	0 22	-0 20	8 54	0 39	8 93	11 91	+2 98	12 3(1)	3 2	39	Phosphate ingestion
VI											10 7(1)	3 1	33	

\* Intakes could not be obtained in period III because of vomiting  
† The arabic numeral in the parenthesis indicates on which day of the period,—first, second, or third,—the blood specimen was collected

During the two control periods the expected findings were present. The serum calcium was very high (14.2 mgm per 100 cc.) the serum phosphorus was very low (2.32 mgm per 100 cc.), the calcium in the urine was markedly elevated (706 mgm in period I compared to a normal

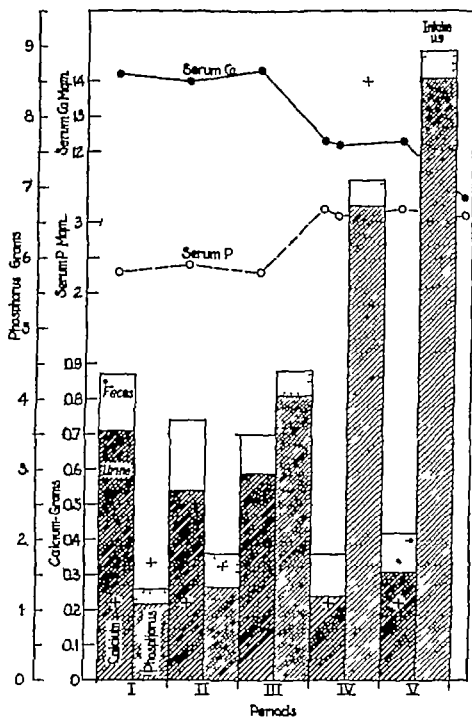


CHART II CALCIUM AND PHOSPHORUS DATA FOR EXPERIMENT TWO

Heavily hatched and dotted columns indicate Ca excretion, lightly hatched and dotted columns indicate phosphorus excretion. Hatched area urinary excretion, dotted area fecal excretion. + indicates intake.

value of 190 mgm for normal individuals under a similar regime (9)), and the phosphorus excretion was predominantly in the urine. The very low fecal calcium excretion alone demands special comment. It would appear that in hyperparathyroidism not only is there no increased excretion of calcium into the gut as a result of the high serum calcium, but there is an increased absorption of calcium from the gut. It is very



significant that in no one of the 46 three-day periods on 13 normal individuals reported by Bauer, Albright and Aub (9) was the fecal calcium excretion on a similar low calcium diet below 200 mgm. Apparently in hyperparathyroidism, calcium is withdrawn from the gastro-intestinal tract as well as from the bones. The low partition of fecal phosphorus compared with urinary phosphorus in this condition probably is dependent on the same principle. This particular finding in clinical hyperparathyroidism does not correspond with the observation made by Albright, Bauer, Ropes and Aub (10) that administration of parathyroid hormone to normal individuals is without effect on the fecal calcium and phosphorus excretions. The discrepancy may be due to the depletion of reserve supplies of calcium in the bones in the long standing cases. Such an explanation is supported by the fact that the fecal calcium continued low in a case of hyperparathyroidism recently studied even after removal of the parathyroid tumor, again suggesting that the decalcification rather than the degree of hyperparathyroidism is the deciding factor here.<sup>2</sup>

With the ingestion of phosphate in periods III, IV, and V, it is very remarkable that the fecal phosphorus excretion was not increased, showing that the additional ingested phosphate was completely absorbed. This again emphasizes the tendency in this condition to absorb calcium and phosphorus from the gastro-intestinal tract. The absorbed phosphorus immediately appeared in the urine. Again there was a rise in the serum phosphorus. One notes that during periods IV and V there was no further rise in serum phosphorus. This probably indicates that the serum phosphorus was now sufficiently above the threshold for phosphorus excretion so that as much phosphorus was leaving the blood through the urine and into the bones as was being absorbed from the gastro-intestinal tract. It is possible that some of the ingested phosphate of the last day of the investigation was still in the gastro-intestinal tract, thus explaining the otherwise unaccountably high balance of phosphorus in period V. As the serum phosphorus rose, the serum calcium fell but again not proportionately and there was a slight rise in the  $\text{Ca} \times \text{P}$  product. The last serum values on the morning following the last day of the experiment are very instructive. We have previously explained the high  $\text{Ca} \times \text{P}$  products with ingestion of phosphate on the ground that phosphorus was being so rapidly absorbed into the blood stream that equilibrium could not be established. Here in period V this absorbed phosphorus was being entirely excreted or deposited. It is not surprising, therefore, at the end of period V to find that the serum calcium had adjusted itself to the then stationary serum phosphorus level and that the  $\text{Ca} \times \text{P}$  product was the same as at the beginning. Finally, with the

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<sup>2</sup> To be published

lowering of the serum calcium, the urinary calcium excretion was diminished

Experiment II very much strengthens the observations made in experiment I, and suggests the following additional conclusions

*VII In hyperparathyroidism there is either a decreased excretion into or more likely an increased absorption of calcium and phosphorus from the gastro-intestinal tract*

*VII-A It follows as a corollary that calcium excretion into the gastro-intestinal tract is not a threshold phenomenon in the sense that calcium excretion into the urine is*

*VIII The changing serum calcium and phosphorus values brought about by phosphate ingestion in hyperparathyroidism reach stationary levels at a new equilibrium when the serum phosphorus has risen sufficiently above the urinary threshold to cause the additional absorbed phosphorus to be excreted in the urine and when the serum calcium has adjusted itself to this new serum phosphorus level*

### *Experiment III*

The subject of this investigation is extremely interesting in that she presents all the metabolic criteria of hyperparathyroidism, but in a milder degree than in any case yet reported. Most cases in the literature have had extreme degrees of this malady. The case history and clinical findings appear in the appendix.

The calcium and phosphorus data are given in Table III and Chart III.

During the control periods all the metabolic abnormalities of hyperparathyroidism were present: the high serum calcium (12.9 mgm per 100 cc.), the low serum phosphorus (2.6 mgm per 100 cc.), the high urinary calcium values, the very low fecal calcium values, and the high partition of phosphorus in the urine. With the ingestion of phosphate one notes again almost complete absorption. This time, however, the increase in the excreted phosphate plus the increase in what phosphate was apparently deposited in the bones was sufficient to account for all the increase in absorbed phosphate so that there was no increase in the serum phosphorus level. One would have expected, therefore, no lowering of the serum calcium and there was no lowering. Thus far everything fits previously discussed ideas.

But the unexpected finding is that the urinary calcium excretion is diminished to a normal level, in spite of no decrease in the serum calcium level. Obviously, some of the calcium which would have been excreted in the urine has been deviated into the bones together with the deposited phosphate. It is evident that one is confronted here with a high serum calcium and a normal urinary calcium excretion, an entirely unprecedented situation in our experience which weakens the conception of calcium as a threshold substance. We can merely point out this dis-

TABLE III  
*Calcium and phosphorus data of experiment III*

Period	Calcium (per 3 day period)					Phosphorus (per 3-day period)					Serum			Remarks
	Urine	Feces	Total excretion	Intake	Balance	Urine	Feces	Total excretion	Intake	Balance	Ca	P	Ca X P	
	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	mgm per 100 cc	mgm per 100 cc		
I	0.63	0.12	0.75	0.22	-0.53	1.53	0.20	1.73	1.68	-0.05	12.9(2)†	2.6	34	Control period
II	0.61	0.17	0.78	0.22	-0.56	1.55	0.28	1.83	1.68	-0.15	13.3(3)	2.7	36	Control period
III	0.62	0.18	0.80	0.22	-0.58	1.31	0.41	1.72	1.68	-0.04	12.1(1)	2.7	33	Control period
IV	0.33	0.12	0.45	0.22		2.61	0.37	2.98	*		12.9(1)	2.8	36	Phosphate ingestion
V	0.22	0.13	0.35	0.22	-0.13	7.12	0.47	7.59	8.48	+0.89	13.3(1) 13.3(2)	2.6 2.8	35 37	Phosphate ingestion
VI	0.17	0.09	0.26	0.22	-0.04	10.26	0.61	10.87	11.88	+1.01	13.1(1)	3.0	39	Phosphate ingestion

\* Intakes could not be obtained in period IV because of vomiting

† The arabic numeral in the parenthesis indicates on which day of the period,—first, second, or third,—the blood specimen was collected

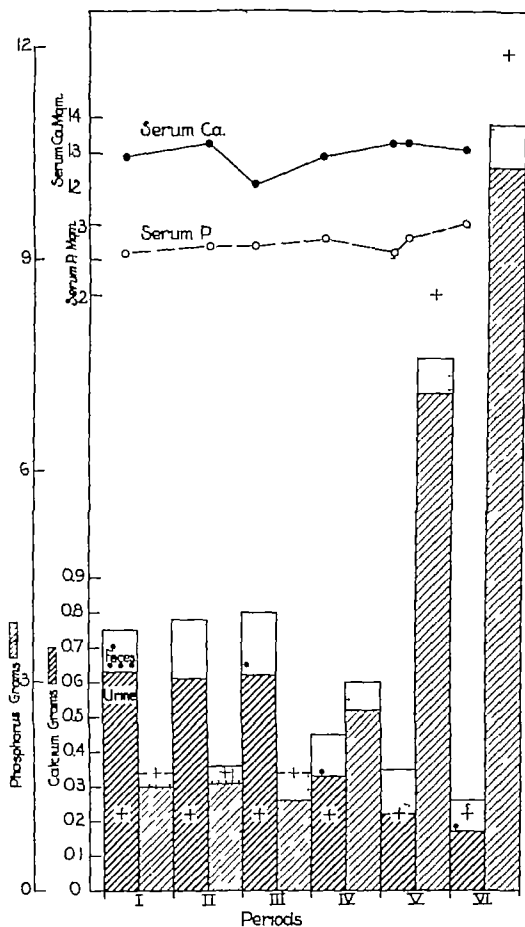


CHART III CALCIUM AND PHOSPHORUS DATA FOR EXPERIMENT THREE

Heavily hatched and dotted columns indicate Ca excretion lightly hatched and dotted columns indicate phosphorus excretion Hatched area urinary excretion dotted area fecal excretion + indicates intake

crepancy but cannot explain it. One might in this connection call attention to the hypercalcinuria with normal blood calcium seen in hyperthyroidism (11), and acidosis (10). These latter instances, although in certain respects the antitheses of the above, do not necessarily test the threshold theory. They merely illustrate that the kidneys can excrete large amounts of calcium without having a readily detectable increase in the serum calcium.

Experiment III, in addition to lending support to previous conclusions, suggests that

*IX Unless the ingestion and absorption of phosphate is so great in hyperparathyroidism that the sum of the increased urinary phosphorus excretion and the increased deposition of phosphorus in the bones cannot keep pace with the increased absorption of phosphorus, there is no rise in serum phosphorus and consequently no fall in serum calcium*

*X It is possible that under certain conditions such as an excess of phosphate for deposit, there may be, in spite of a very high serum calcium, a normal calcium excretion in the urine*

## DISCUSSION

### *A Dangers of phosphate administration*

By way of warning, we believe that phosphate ingestion in hyperparathyroidism is associated with two real dangers.

In the first place it has been shown by animal experimentation (12) that before death from parathyroid overdosage there is a shutdown of kidney function, presumably due to the increased viscosity of the blood, and a resulting rapid rise in serum nonprotein nitrogen and inorganic phosphorus. When this complication occurs there is present in the blood a high serum calcium and high serum phosphorus. One is then confronted with our second group of disorders of calcium phosphate metabolism, namely, a supersaturation of the blood with calcium phosphate and a tendency to precipitate calcium phosphate into tissues other than osteoid tissue. If parathormone administration is continued, the dogs die in uremia and show at autopsy calcium deposits in the alveolar walls of the lungs, the mucosa of the stomach, the kidney parenchyma, and the thyroid gland (13). Any administration of phosphates in the presence of this complication, this state of parathyroid poisoning as it were, would merely hasten death by increasing this supersaturation of the blood. On this ground we believe the administration of phosphate to a patient at a time when the serum calcium and phosphorus are both high is contraindicated. The patient with multiple myeloma reported by Bulger, Dixon, Barr, and Schregardus (14) may be a case in point. This patient had a blood calcium of 17.8 mgm and a phosphorus of 5.3 mgm when phosphate was administered and showed at autopsy calcium

deposits in the lungs, gastric mucosa, and kidneys. It is of interest that most of the cases of hyperparathyroidism reported are of a severity which would be considered near the danger level of parathyroid poisoning in animals. It is surprising that they do not surpass this level more often and reach the state of parathyroid poisoning and death. There are three such cases in the literature where death was probably due to parathyroid poisoning, Dawson and Struthers (41), Hoffheinz (15) and Penecke (16). These patients died in uremia and showed at autopsy in addition to the osteitis fibrosa cystica generalisata and parathyroid tumors, calcium deposits in the lungs, thyroid, kidney parenchyma, and other organs. They were therefore, pathologically analogous to dogs dying from parathormone overdosage (13).

The second danger of phosphate ingestion in these patients is more often encountered. Many of these patients have bilateral calcium phosphate stones in the kidney pelvis. Thus, patient I gave the history of having passed gravel and patient II has bilateral kidney stones. The stones are without doubt due to the increased calcium and phosphorus in the urine. Phosphate ingestion increases tremendously the phosphaturia and hence the danger of stone formation. The decreased calcinuria is of course, a favorable factor. It seems wise in administering phosphate to force fluids and to keep the urine acid. Mono-sodium phosphate  $s$ , therefore, perhaps preferable to the more alkaline salts.

### *B Secondary hyperparathyroidism*

In the introduction we have summarized our conception of the *modus operandi* of the parathyroid hormone in producing changes in Ca and P metabolism. We have suggested that the first step is an apparent change in the threshold for phosphorus excretion in the urine. The evidence for a high threshold in hypoparathyroidism is twofold: (a) The almost complete disappearance of phosphorus from the urine in a case of hypoparathyroidism on an extremely low phosphorus diet in spite of a continued high serum phosphorus (2) and (b) the promptness with which the phosphorus excretion in the urine dropped when a high phosphorus diet was suddenly changed to a low phosphorus diet in the same individual, showing that the high serum phosphorus did not represent a renal retention phenomenon.

The evidence for a low threshold for phosphorus excretion in hyperparathyroidism is: (a) The promptness with which absorbed phosphate is excreted in the urine in the patients here discussed and (b) the resistance which the low serum phosphorus exhibited to being raised by the large amounts of phosphate which passed through the blood in these experiments.

With these facts before us a previously unaccountable finding in a patient studied by Aub, Albright, Bauer and Rossmesl (17) perhaps now

becomes explainable and very instructive. This patient because of chronic steatorrhea and the consequent lack of absorption of calcium had reached a condition where the serum calcium was very much diminished. The serum phosphorus was likewise reduced. The disorder of the calcium phosphate metabolism was, therefore, of the "subsaturation" variety. The unexpected finding was that in spite of the low serum phosphorus this patient was excreting a normal or even increased amount of phosphorus in the urine. The supposition had to be made that, if such a thing as a kidney threshold for phosphorus exists, it must have been lowered in this patient. But, having used lowering of kidney threshold for phosphorus to explain the findings in hyperparathyroidism, one did not like to resort to this in an entirely different situation. But was the situation entirely different?

Is there any evidence that hyperparathyroidism may exist as a secondary phenomenon in the "subsaturation" group of disorders? In 1907 Erdheim (18), then a pupil of Weichselbaum, studied the parathyroid glands in seven cases of puerperal osteomalacia and one case of senile osteomalacia. In six of the eight cases the parathyroid glands were either enlarged or showed evidence of hyperplasia. In five of the six cases multiple parathyroid glands were involved. The histological evidence for hyperplasia rested on the presence of many circumscribed zones of young cells which were akin to the chief cells but which with fat stains showed very little intracellular fat. We can refer to these as proliferation zones (Wucherungsherde). In only one of nine control cases did Erdheim find the same degree of histological evidence of hyperplasia and this was a very old man whose skeleton was not examined. In 1914 Erdheim (19), by an ingenious projection method, made enlarged wax models of parathyroid tissue in rats and was able to show that hypertrophy of all parathyroid tissue including accessory parathyroid tissue occurs in rats suffering from rickets. The World War interrupted his measuring the parathyroids in cases of infantile rickets in the same way. He made the preliminary observation, however, that the parathyroids appear enlarged in this condition, although microscopically he was unable to note any constant changes. Bauer (20), also a pupil of Weichselbaum, reported in 1911 a case of osteomalacia in which the bone condition was noted only after attention had been directed to the skeleton by the finding of hypertrophy and hyperplasia in all the parathyroids. Weichselbaum (21) himself reported the finding of enlarged parathyroids in two cases of late rickets. The findings of this Viennese school have on the whole been substantiated.

Thus, in 1907 Schmorl (22) reported parathyroid hypertrophy in only one of four cases of osteomalacia and failed to find parathyroid changes in four cases of rickets and two cases of late rickets, but neglected to study the parathyroids with fat stains for proliferation zones, in 1909 Strada

(23) reported hyperplasia in one case of osteomalacia in 1912 Todyo (24), a pupil of Schmorl, reported hyperplasia in six out of seven cases of osteomalacia and in eight out of eleven cases of senile osteoporosis, and in only four out of twenty four control cases two of these four being in pregnant patients, a condition which in itself brings about hyperplasia (25), in 1912 Hohlbaum (26) reported a case of osteomalacia with hypertrophy and hyperplasia of all parathyroid glands in 1916 Maresch (27) reported hypertrophy and hyperplasia of the parathyroids in eight cases of senile osteomalacia and twenty eight cases of senile osteoporosis in 1920 Ritter (28) reported hyperplasia of the parathyroids in ten cases of rickets, three cases of osteomalacia and one case of osteoporosis in 1921 Pappenheimer and Minor (29) found the parathyroid glands in fourteen cases of rickets much larger than those from eighteen non rachitic controls in 1922 Hartwich (30) found hypertrophy of the parathyroids in fifteen cases of rickets, in 1925 Kerl (31) found hypertrophy and hyperplasia of all parathyroids in a case of puerperal osteomalacia and a case of osteoporosis in the same year Danisch (32) found proliferation zones of chief cells in the parathyroids in twenty three out of forty seven individuals over sixty years and out of these nineteen had senile osteomalacia in 1925 Doyle (33) found the parathyroids of rachitic chickens enlarged in 1926 Nonidez and Goodale (34) confirmed this and in 1928 Higgins and Sheard (35) showed that ultraviolet light prevented the hypertrophy of chickens parathyroids on a rachitic diet

We have therefore, pathological as well as metabolic evidence that the parathyroid glands are overactive in the 'subsaturation' group of disorders. It should be emphasized that multiple parathyroids are involved in this condition and that as Erdheim first pointed out there is every reason to believe this a compensatory mechanism. The situation is in direct contrast to primary hyperparathyroidism, associated with *osteitis fibrosa cystica generalisata* in which only one parathyroid is enlarged but this to a marked extent. It seems entirely probable that the stimulus for the parathyroid gland secretion is a low blood calcium, that a low blood calcium, when dependent on conditions outside of the parathyroid glands produces a hyperplasia of the glands that such conditions are, consequently, associated with a secondary hyperparathyroidism and that, therefore, a low threshold for phosphorus excretion is to be expected. The presence of hyperparathyroidism with a low blood calcium is after all not so surprising as we are dealing with hyperparathyroidism complicating the "sub rather than the "normal saturation' state. According to the theory outlined here the reason for the high blood calcium in hyperparathyroidism is to compensate for the low blood phosphorus in order to maintain normal saturation, but in the subsaturation group of disorders all possibility of compensation has been lost. Further evidence of the correctness of this view is given in un



published data obtained by Bauer and Marble. They were unable to elevate the depressed serum calcium in this same patient with parathormone. This could have been and as a matter of fact was predicted. Furthermore, when this underlying difficulty, the failure to absorb calcium, was overcome by ergosterol medication, the serum calcium promptly rose, but the serum phosphorus lagged behind for many weeks and was the last abnormality to correct itself (36). One would expect the hyperplasia to disappear only gradually when the cause of the hyperplasia, the low serum calcium, had been removed.

A lowering of the blood phosphorus without a raising of the blood calcium by the parathyroid hormone in the subsaturation group of disorders is not without example in the literature. Shohl, Wakeman, and Shorr (37), studying the effect of parathormone on infantile tetany, produced in one of two cases a fall in serum phosphorus from 9.3 to 3.1 with no corresponding rise in serum calcium. Our metabolic criteria of hyperparathyroidism, namely a high urinary P excretion in the presence of a low serum P, were present in the patient with osteomalacia studied by Gargill, Gilligan, and Blumgart (38) and in the four patients with osteomalacia studied by Miles and Feng (39).

The implication of such a theory, which states that disorders associated with subsaturation may be, in fact probably usually are, associated with secondary hyperparathyroidism is far reaching. It means that part of the disordered metabolism of rickets and osteomalacia is due to secondary hyperparathyroidism. It means that the blood calcium is no longer the measuring stick of the degree of hyperparathyroidism, rather the blood phosphorus. It means that, although in osteitis fibrosa cystica generalisata the bone condition is secondary to the parathyroid condition, in rickets and osteomalacia, and other allied conditions the parathyroid abnormality is secondary to the bone abnormality.

That the abnormalities in calcium metabolism in hyperparathyroidism are minimized by phosphate ingestion and that these findings support the theory of parathyroid function which makes the calcium abnormalities secondary to the phosphorus abnormalities has been clearly demonstrated and amply discussed. We did not try phosphate ingestion on the one case of proven parathyroid tumor which we have had the opportunity to study in our clinic. That such patients, however, respond in a similar fashion is shown by the experiments of Bulger, Dixon, Barr and Schregardus (14). Their patient I belonged in this group and before operation phosphate ingestion produced changes similar to those reported in this paper. There is one very definite difference, however, between the sequence of events which follows the removal of a tumor in hyperparathyroidism and that which follows the ingestion of phosphates. In the former case, the phosphorus rise and the calcium fall in the serum are such that the product of  $\text{Ca} \times \text{P}$  does not rise, in fact temporarily often

falls. In the latter case, we have observed that the product rises. Thus, whereas the calcium fall is obviously due to the phosphorus rise in the latter case, if the same is true in the former, there is left to be explained why in the former case the calcium fall is more delicately adjusted so as not to disturb the  $\text{Ca} \times \text{P}$  product. Whereas this finding is against the theory we are supporting we believe it can be explained. The explanation will be reserved until a later paper which discusses the sequence of events in calcium and phosphorus metabolism following the removal of a parathyroid tumor.

### SUMMARY AND CONCLUSIONS

1 Three cases of clinical hyperparathyroidism have been treated by ingestion of phosphates with beneficial results.

2 The metabolic sequelae of such treatment are (a) almost complete absorption of phosphates into the blood stream (b) rapid excretion of the absorbed phosphates by the kidneys (c) a rise of the previously low serum phosphorus, (d) a fall of the previously elevated serum calcium (e) a rise at first of the  $\text{Ca} \times \text{P}$  product in the serum, and (f) a fall of the urinary calcium excretion.

3 The net result is an increase in the balances of calcium and phosphorus and a return of the serum levels of both in the direction of normal.

4 The rapidity with which the absorbed phosphorus is excreted in the urine and the resistance which the low serum phosphorus exhibits to being raised suggest that the threshold for phosphorus excretion into the urine is lowered in hyperparathyroidism, and we consider this the most fundamental change in the Ca and P metabolism in this condition.

5 It appears that in long standing hyperparathyroidism there is increased absorption of calcium and phosphorus from the gastrointestinal tract and that excretion of calcium into the gastrointestinal tract is not a threshold phenomenon dependent on the level of serum calcium.

6 There are two theoretical dangers of phosphate ingestion in hyperparathyroidism (a) precipitation of the state of parathyroid hormone overdosage called in this paper "parathyroid poisoning, with death from uremia and calcium deposits in the lungs, mucosa of the stomach, thyroid gland and kidney parenchyma (b) danger of producing bilateral phosphate stones in kidney pelvis.

7 It is suggested that a low serum calcium is the stimulus for the parathyroid secretion and that a low serum calcium due to causes other than parathyroid hypofunction leads to secondary hyperparathyroidism.

8 Inasmuch as conditions other than hypoparathyroidism associated with a low serum calcium for the most part belong to the "subsaturating group" and inasmuch as a rise in serum calcium in response to the parathyroid hormone would only be expected to occur under conditions of

normal saturation, secondary hyperparathyroidism is often not associated with hypercalcaemia

9 It follows as a corollary that the serum calcium is not always a measuring stick of the degree of hyperparathyroidism,—rather the serum phosphorus

10 A review of the literature adds both histological and metabolic evidence to support the existence of secondary hyperparathyroidism in the “subsaturation” group of disorders

## APPENDIX

*Case 2* Mrs N B (M G H number 307624), a married white woman of 41 entered the Massachusetts General Hospital on July 11, 1930, complaining of rheumatism of 5 years' duration

The *history* showed the following salient features 5 years ago, following fifth pregnancy, onset with generalized weakness and pains in knees 3 years ago exacerbation during eighth month of sixth pregnancy Crutches then became necessary She was treated for flat feet and teeth were removed without benefit Two years ago she slipped and fractured right hip, requiring four months in plaster cast The fracture united Seven months ago, she slipped and fractured collar bone on right and shortly thereafter without adequate cause she fractured the upper end of her right humerus Patient had had polydipsia for about two years No abnormality of diet was elicited Calcium and phosphorus intakes were not deficient

The *past history* was entirely irrelevant The catamenia was not remarkable

*Physical examination* A rather obese woman slightly bent over, mentally clear and very intelligent Pupils reacted to light and in accommodation Mucous membranes were slightly pale Tonsils were small and buried All teeth were out and no false teeth had been supplied Neck showed no tumor Left border of heart was 1 cm beyond mid-clavicular line A systolic murmur was present at the apex Blood pressure was 185 systolic and 105 diastolic Lungs not abnormal Liver and spleen were not felt Knee jerks present and sluggish There was tenderness over ribs and long bones and left shin bone felt rough and nodular

*Laboratory findings* Blood—Red count 3,400,000 White count 4,800 Hemoglobin 60 per cent Smear was not remarkable Urine showed albumin and clumps of white cells in all specimens Cultures showed no growth Gastric analysis—Free hydrochloric acid was present after ergamine Basal metabolism—+ 3 Wassermann—negative Serum calcium, 14.2 mgm per 100 cc Serum phosphorus, 2.3 mgm per 100 cc

*Cystoscopic report* (Dr C S Swan) "The pvelograms show bilateral hydronephrosis with injection. The functions and nonprotein nitrogen have not been materially affected to date. The stone in the left kidney is in the renal pelvis and acts probably as a ball-valve."

*X rays* These have been reported in detail by Dresser and Hampton (40). All the bones examined are abnormal; they appear coarse in texture and of increased radiability. The bone trabeculae are widely separated, interrupted, and disorganized. There are multiple cyst-like areas of bone destruction, most numerous in the pelvis and upper ends of the femora where they vary from 1 to 3 cm in diameter. These cysts are sharply defined and are seen in the pelvis, left radius, ribs, both bones of both lower legs, skull and scapulae. In the ribs, radius, and fibulae, there is expansion and thinning of the cortex of the bone simulating a giant cell tumor. There is evidence of pathological fracture in the right fibula and crest of the left ilium. These fractures have united with an overgrowth of bone. The skull and vertebrae present a peculiar moth-eaten appearance, characterized by punched out areas of bone absorption intermingled with dense round bone deposits. These small bone deposits are about 2 mm in diameter and the areas of bone destruction vary from 1 cm down to 1 mm. In addition the x rays showed bilateral renal calculi.

*Operation* (Dr Richard C Miller) The thyroid was completely exposed but no parathyroid tumor could be found. The two lower parathyroids were identified and removed. They were each about the size of a split pea. The upper parathyroids were not seen. Some tissue was removed in addition which later turned out to be thymus.

*Microscopic examination of parathyroids* revealed "structure of normal parathyroid glands."

*Result of operation* was without benefit to patient.

*Case 3*, Mrs M D (M G H number 276450), a married colored woman of 22, first entered the hospital on May 21, 1926, because of pain in the right hip of two years' duration.

*Present illness* Patient had been married 2½ years. During this time she had had four pregnancies, the first two resulting in miscarriages, the third resulting in the birth of a child which lived four weeks, and the fourth terminating three weeks before entry with the birth of a healthy son.

For over two years patient had had pain in her right hip which was aggravated by pregnancies. During past five months patient had been unable to flex thigh or abdomen because of pain. There had been recently pains in ribs as well, made worse by deep breathing.

*Past history* Mumps, measles, and pertussis in childhood. Scarlet fever at 4 years, typhoid at 8 years. "Pneumonia" three times, the last at 9 years of age. Catamenia had always been irregular and she often

skipped periods. She had lost from 105 pounds her best weight to 87 pounds during the past 4 years.

*Physical examination* A very small colored girl in fair state of nutrition. Breasts were distended with milk. Teeth showed pyorrhea but otherwise appeared strong. Tonsils enlarged. Lungs negative. Ribs were painful to pressure. Tenderness of sacrum by rectal. Right hip motion (rotation) limited. There was transmitted tenderness to either

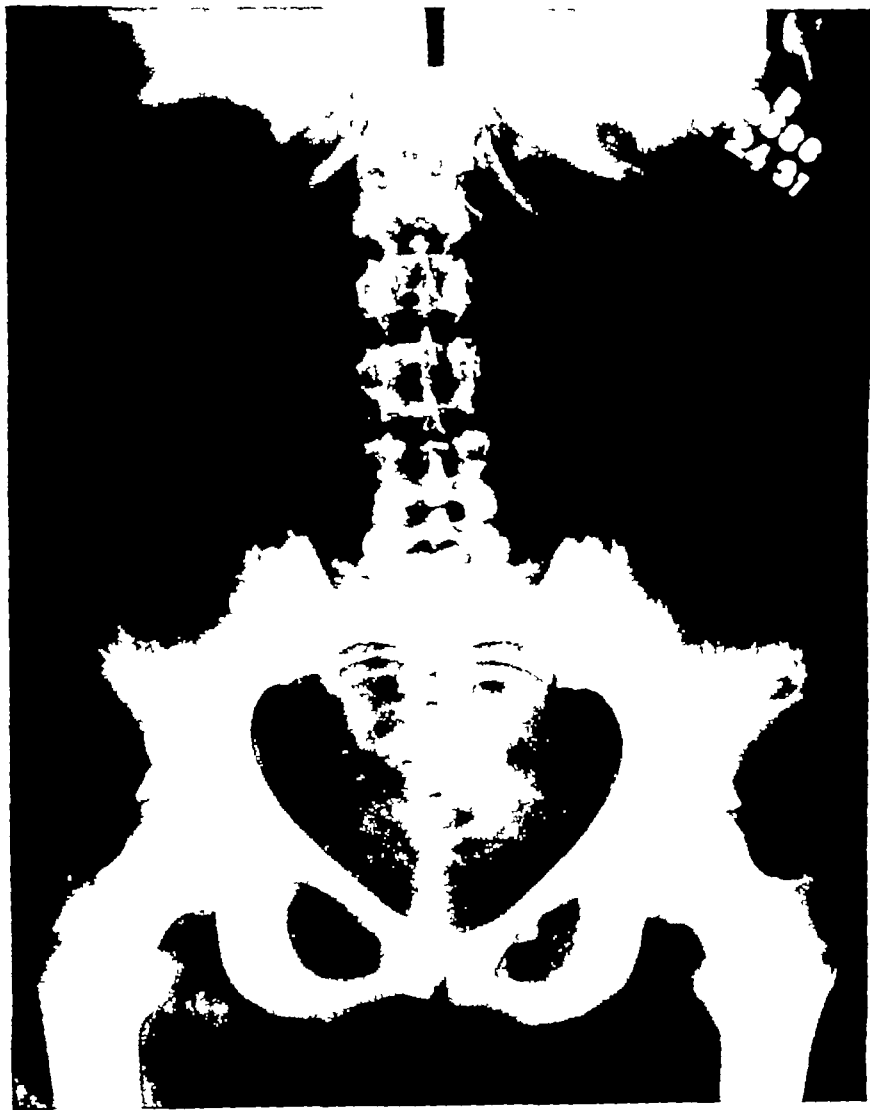


FIG. 1 X-RAY OF PELVIS IN MRS. M. D., PATIENT THREE

hip on percussion of opposite hip. She held right leg flexed and everted. Blood pressure was 90 systolic and 35 diastolic.

*Laboratory findings* Urine not remarkable. No Bence-Jones protein. Red count 3,600,000. Hemoglobin 60 per cent. White count 6,800. Wassermann negative.

$\lambda$  rays (Interpreted by Dr George W Holmes) Chest showed increase in hilus shadows Plate taken of the lumbar spine and pelvis shows multiple areas of diminished density in the bones of the pelvis spine, ribs, and the upper end of the femur These areas are ring shaped and probably represent areas of destruction in the bone There is very little if any evidence of reaction around them All bones are less dense than normal Skull shows characteristic appearance of osteomalacia " Teeth  $\lambda$  rays showed apical abscesses

*Diagnosis* Osteomalacia

*Treatment* X ray sterilization and high calcium diet and cod liver oil

*Subsequent history* Patient again entered the hospital on June 16, 1930 for check up The symptoms related to her skeleton had disappeared Her only complaint was hot flashes and amenorrhea of 4 months duration, both attributable to  $\lambda$  ray sterilization Serum calcium was 13.0 mgm per 100 cc and serum phosphorus 2.4 mgm per 100 cc X-rays of the bones at this time showed undoubted improvement although the previous  $\lambda$  rays were not available for comparison The scattered areas of diminished density were still apparent in the pelvis (see Figure 1) At this time studies here reported were performed

*Final diagnosis* Mild hyperparathyroidism

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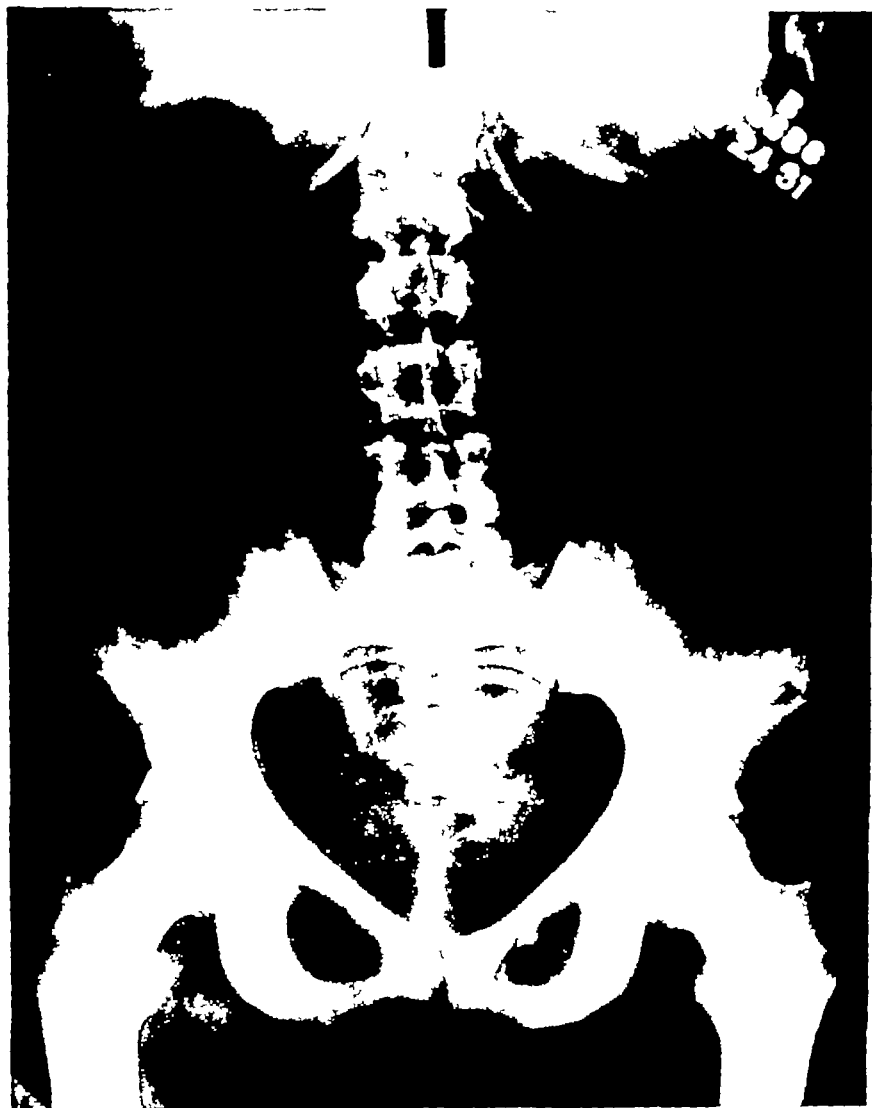


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# THE SPECIFIC DYNAMIC RESPONSE TO PROTEIN OF INDIVIDUALS SUFFERING FROM DISEASE OF THE HYPOPHYSIS

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Many attempts have been made to assign to the hypophysis the regulation of the increase in heat production which follows the ingestion of a protein meal, just as the thyroid gland appears to regulate the level at which the basal metabolic rate is set

Plaut (1) in 1922 studied the response of groups of individuals to a standard meal consisting of 200 grams of chopped meat 50 grams of fat 200 grams of bread and 500 cc of coffee She found that the maximal increase in heat production that resulted from this meal occurred 45 minutes after its ingestion was maintained through the second hour, and was definitely decreasing in the third The increase in heat production above the basal value in normal individuals was 24 to 52 per cent In what she described as 'hypophyseal obesity' (2) the response varied from  $-3$  to  $+18$  per cent She stated that a normal basal metabolic rate coupled with a lowered specific dynamic response to protein, is characteristic of disease of the hypophysis and therefore of great diagnostic value Plaut (2) (3) (4) Plaut and Schadow (5) and Kestner, Plaut, and Schadow (6) have reported many determinations in confirmation of this earlier work Similar results have been obtained by Knipping (7) Liebesny (8) fed a similar meal (200 grams meat 100 grams bread) to a group of normal individuals, 4 dwarfs, 2 acromegalics, 1 case of hypophyseal tumor 4 cases of infantilism and individuals suffering from conditions related to 'disturbances of the autonomic nervous system' He determined the increase in heat production 2 hours after the ingestion of the test meal and concluded that the specific dynamic response of the normal individual to the above meal is 30 per cent above the basal with a variation of  $\pm 10$  per cent A decrease or complete absence, occurred in disturbances of the pituitary body (hypophyseal dwarfs hypophyseal tumor), obesity, especially of hypophyseal origin and in conditions related to disturbances of the autonomic nervous system (Raynaud's disease scleroderma) Further he found that the administration of a preparation of anterior lobe of the hypophysis resulted in an increase in the specific dynamic response In a later paper (9), he reports that diathermy of the hypophysis of individuals suffering

from disturbances of the sex glands resulted in an appreciable increase in their specific dynamic response. Bernhardt (10) found a low specific dynamic response in conditions other than disturbances of the pituitary body. He believes that it is possible that some relationship exists between the hypophysis and the increase in heat production, following a protein meal, but that other endocrine glands may be concerned as well. A similar position is taken by Gantenberg (11), in a very extensive review of the clinical significance of determinations of the specific dynamic response.

The experiments carried out by Plaut and her associates have been criticized with regard to the meal fed and the technique used. Pollitzer and Stolz (12) found that the maximal increase in heat production resulting from the ingestion of 250 grams of ground meat alone occurred in the third to fifth hour. They further noted that if the heat production was determined only one to two hours after the ingestion of the meat, four-fifths of the subjects they studied gave negative results. They attribute the early increase observed by Plaut to the large amount of carbohydrate in the test meal. Plaut (4) herself answers these criticisms by asserting that the ingestion of fairly large amounts of carbohydrate with meat favor rapid emptying of the stomach, so that the specific dynamic response is observed more quickly than when meat alone is fed. Durr (13) makes the same criticism of the work of Plaut, and points out that an individual without any evidence of hypophyseal disease exhibited no significant specific dynamic response in three hours, and only 18 per cent in five hours. Lublin (14) has questioned the technique used by Plaut. He feels that she obtained too great variation in the basal metabolic rate in normal individuals, and points with skepticism to several fasting respiratory quotients greater than one. He, likewise, feels that a single determination of the heat production one to two hours after the test meal is not sufficient for generalization. From his own work he concludes that a low specific dynamic response is not diagnostic of pituitary disease. Jaguttis (15) came to the same conclusion. Lauter (16) in a careful study of obesity, points out that the specific dynamic response of normal individuals to protein varies within very wide limits, and that since the normal variations are so large abnormalities can be postulated only if no increase at all results from the ingestion of protein.

protein, which was returned to normal in both cases by the administration of an anterior lobe preparation (Präphvson) Foster and Smith (18) found that rats after the removal of the hypophysis exhibited no specific dynamic response to the administration of glycine, and that the restoration of the normal response occurred only if extracts of both anterior and posterior lobe were simultaneously injected. Contrary results, however, were obtained by Gaebler (19), working in Lusk's laboratory, who found that dogs, from whom the hypophysis had been removed, responded normally to a standard protein meal.

It is clear that no agreement exists among investigators in regard to the relationship between the pituitary body and the specific dynamic action. Part of the confusion, no doubt, arises from laxity in the selection of material. Consequently, we have restricted our studies to patients in whom physical abnormalities of the hypophysis had been certainly demonstrated.

### PROCEDURE

It seemed desirable to eliminate the effect of ingested carbohydrate. Accordingly the test meal consisted of 300 grams of sirloin steak, freed from all extraneous fat, ground and fried in a small amount of butter. The subject was allowed to drink water with the meat. In the first subject studied 700 grams of meat were fed on two different occasions, but this amount was too large to be eaten readily. The basal metabolic rate was determined before the meat was given, and the heat production again measured at the intervals recorded in Table I. When a significant increase in heat production was observed under satisfactory conditions, the experiment was discontinued. Determinations were carried out by means of a Tissot spirometer, and the gas analyses were made with the Henderson apparatus.

The significant physical findings in each case are recorded in the appendix.

### RESULTS

Examination of Table I makes it clear that each of the six subjects studied, responded to the ingestion of 300 grams of meat, with a definite increase in heat production. The maximal responses to the same meal varied from 18 to 28 per cent above the basal.

Case number 248006 was studied on October 16, 1930, and a response of only 12 per cent was observed, although his heat production was followed for five hours after the ingestion of the meat. Opportunity was given us, however, to repeat this determination. This time, in the second hour, his heat production was 28 per cent above the basal level. It is probable that a true basal value was not obtained in the first experiment. If we recalculate this experiment in terms of the second basal, we find an increase in heat production of 21 per cent in the first experiment.



from disturbances of the sex glands resulted in an appreciable increase in their specific dynamic response. Bernhardt (10) found a low specific dynamic response in conditions other than disturbances of the pituitary body. He believes that it is possible that some relationship exists between the hypophysis and the increase in heat production, following a protein meal, but that other endocrine glands may be concerned as well. A similar position is taken by Gantenberg (11), in a very extensive review of the clinical significance of determinations of the specific dynamic response.

The experiments carried out by Plaut and her associates have been criticized with regard to the meal fed and the technique used. Pollitzer and Stolz (12) found that the maximal increase in heat production resulting from the ingestion of 250 grams of ground meat alone occurred in the third to fifth hour. They further noted that if the heat production was determined only one to two hours after the ingestion of the meat, four-fifths of the subjects they studied gave negative results. They attribute the early increase observed by Plaut to the large amount of carbohydrate in the test meal. Plaut (4) herself answers these criticisms by asserting that the ingestion of fairly large amounts of carbohydrate with meat favor rapid emptying of the stomach, so that the specific dynamic response is observed more quickly than when meat alone is fed. Durr (13) makes the same criticism of the work of Plaut, and points out that an individual without any evidence of hypophyseal disease exhibited no significant specific dynamic response in three hours, and only 18 per cent in five hours. Lublin (14) has questioned the technique used by Plaut. He feels that she obtained too great variation in the basal metabolic rate in normal individuals, and points with skepticism to several fasting respiratory quotients greater than one. He, likewise, feels that a single determination of the heat production one to two hours after the test meal is not sufficient for generalization. From his own work he concludes that a low specific dynamic response is not diagnostic of pituitary disease. Jaguttis (15) came to the same conclusion. Lauter (16) in a careful study of obesity, points out that the specific dynamic response of normal individuals to protein varies within very wide limits, and that since the normal variations are so large abnormalities can be postulated only if no increase at all results from the ingestion of protein. Therefore, he questions the diagnostic value of such a procedure. Gessler, Kraus, and Rettig (17) also found considerable variation in a series of normals. They believe that no conclusions can be drawn from a small section of the curve, but that the whole course of the reaction must be followed.

Attempts have been made to solve the question by injury, or removal of the hypophysis. Knipping (7) injured the hypophyses of two dogs by the Cushing method, and found a low specific dynamic response to

Recently Dock (30), by excluding from the circulation various portions of the bodies of rats, found that at least 85 per cent of the heat evolved as the specific dynamic action of protein was liberated in the abdominal viscera. He concluded that "the liver is probably the chief site and possibly the only site of the intermediary metabolism of those amino acids which raise the metabolic rate, and that at least 80 per cent of the specific dynamic action of these amino acids is due to the increased energy liberated by the hepatic cells during protein digestion."

### CONCLUSION

1 Six individuals with definite abnormality of the pituitary body responded to the ingestion of a protein meal, with a large increase in heat production

2 Experiments designed to show the dependence of the specific dynamic action of protein upon the secretory activity of the pituitary body are unconvincing. In fact, recent investigation goes far to demonstrate that the two are quite independent

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in the fourth hour after the protein meal. If, however, this experiment had not been repeated it would have been necessary to conclude that here at least was a definitely lowered response to protein. It is evident that these six individuals, each of whom had some involvement of the pituitary body, exhibited a large response to a protein meal.

## DISCUSSION

The diversity of the results obtained by various investigators is not surprising when the difficulties encountered in this field are fully appreciated. In recent times there has been great enthusiasm displayed in attributing disease to endocrine abnormalities. However, it still remains true that a definite diagnosis of pituitary disease should be based on a demonstration of definite physical abnormalities of this organ. If the clinical material thus far presented is examined with such criteria in mind, insufficient cases remain on which to base the published conclusions.

Further, in the case of the "normal" human subject, a uniformity of results comparable to those reported by Lusk (20) for the dog has not been obtained. Missal and Johnston (21) found it impossible to secure a consistent response to ingested sucrose or glycine in a normal subject maintained on a constant diet. Further, Benedict and Carpenter (22) state, "that while the ingestion of protein in almost any amount invariably produces an increase over the basal metabolism (which may be 25 per cent for several hours, and for short periods may rise to 45 per cent), no definite mathematical relationship between the amount of protein ingested and the increment in the total metabolism can be noted from these values."

Further, in critically evaluating the literature it is necessary to bear in mind that evidence exists that the state of nutrition (11) influences the height of the specific dynamic response, and that Coleman and DuBois (23) have shown it to be lowered in fever.

Recent animal experiments suggest that the liver is responsible for the increased heat liberated during protein metabolism. It is not clear whether this extra heat production results from a general increase in oxidative processes throughout the body, stimulated by some intermediary product of protein metabolism, as Lusk (20) has suggested, or whether it results from exothermic reactions involved in the intermediary metabolism of protein, as for example, conversion of amino acids to glucose, as Geelmuyden (24) thinks.

Bollman, Mann, and Magath (25) (26) showed that deamination of the amino acids was prevented by hepatectomy, while Wilhelmj and Mann (27) (28) (29) found that the administration of amino acids to hepatectomized dogs did not increase the metabolism. These authors are inclined to interpret their results as evidence that not the amino acids, but other intermediate products of their metabolism, are the stimulators of cellular oxidation throughout the body.

## APPENDIX

## CASE RECORDS

Hospital number—237024 Date of admission—March 18, 1924  
Sex—female

*History* Seventeen years before admission the patient underwent a subtotal oophorectomy without change in the character of the menses. Thereafter she considered herself well until four years ago, when she began having attacks of unconsciousness without convulsions. Severe headaches became frequent. A pronounced increase in appetite resulted in a gain in weight of 85 pounds. During the past two years there has been an increasing memory defect, accompanied by definite psychopathic tendencies which caused her to be brought to the Psychopathic Hospital.

*Physical examination* Essentially negative, except for pronated feet, and obesity. The neurological examination revealed no physical abnormalities. Kahn test of the blood was negative.

*Ophthalmological examination* The visual fields showed a rather generalized contraction with an achromotopsia.

*X-ray examination* X-ray examination of the skull revealed complete destruction of the pituitary fossa with no evidence of the anterior or posterior clinoid processes.

*Diagnosis* Psychosis with organic brain disease, tumor of pituitary gland. This patient was given deep x-ray therapy with resultant relief of headaches and some improvement in visual fields. Date of discharge—June 17, 1930.

Hospital number—252427 Date of admission—October 30, 1930  
Sex—male.

*History* Patient was brought to the hospital because of attacks of abdominal cramps 2 or 3 times a week, which were relieved by vomiting. He also suffered from visual difficulties, especially upon awakening in the morning, when he could not see objects distinctly. He had been having mild recurring headaches. Patient claimed that his weight increased from about 100 pounds a year ago to about 165 at admission.

*Physical examination* Obese, marked acne about upper part of body. Expression apathetic. Left palpebral fissure narrowed. Kahn test of blood negative.

*Ophthalmological examination* Choking of both discs, 2 diopters. Fields show a definite left temporal defect.

*X-ray examination* Relative destruction of the sella turcica. Depression of the floor and rarefaction of the posterior clinoid processes with diastasis of the main sutures, suggesting severe internal hydrocephalus.

*Operation* Diagnosis of pituitary tumor was made and operation performed February 13, 1931, which consisted of osteoplastic craniotomy.

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olism in the Rat

*History* Patient entered the hospital because of blindness of right eye of about one month's duration, and severe headaches. She had had severe headaches over a period of ten years without other complaints until about one year ago. Since then she has had dyspnea, progressive weakness and dimness of vision. One month ago she suddenly became blind in the right eye.

*Physical examination* The physical examination revealed the standard signs of chronic nephritis with hypertension.

*Ophthalmological examination* There was a hemorrhagic retinitis of both eyes. Fundus examination showed choked disc for both eyes—4 diopters. Fields could not be obtained for the right eye but there was marked evidence of optic atrophy of the left with great restriction of the visual field on this side. Kahn test of the blood was negative.

*X-ray examination* Detailed studies of the sella turcica demonstrate a destruction of the floor of the sella turcica and of portions of both the anterior and posterior clinoid processes. Operation was advised against because of the nephritis. The patient was given deep x-ray therapy. Date of discharge March 6, 1931.

*Diagnosis* Chronic nephritis with hypertension. Tumor of the pituitary gland.

Hospital number—257784. Date of admission—January 31, 1931. Sex—male.

*History* Four years before admission patient began to notice weakness and fatigueability. His physician made a diagnosis of diabetes mellitus. His symptoms were relieved by diet until the past two years, when his symptoms have increased so that he could not work, and he sought relief at the hospital. During the past ten years his wife has noticed a marked alteration in his appearance and temperament. He has changed from an aggressive, interested husband into an irritable and careless one. He himself has noticed a thickening of the lower lip and a thickening and widening of the fingers. He has noticed no visual disturbances.

*Physical examination* Ptosis of left upper eyelid. Thick, drooping lip. Tongue strikingly broad and thick. Huge broad hands with thick bulbous fingers. There was a persistent glycosuria of the diabetic type, which was controllable. The glucose tolerance curve was of the diabetic type. Kahn test of the blood was negative.

*Ophthalmological examination* The visual fields showed definite right homonymous hemianopsia.

*X-ray examination* X-ray studies of the skull showed almost complete destruction of the posterior clinoidal processes and apparent thinning of the floor of the sella turcica. X-ray examination of the hands showed typical changes of acromegaly.

with exploratory puncture into the lateral ventricle that resulted in the escape of a large quantity of fluid. The sella turcica was excavated but there was no tumor of the hypophysis. Patient died February 15, 1931.

*Pathological diagnosis* Suprasellar cystic astrocytoma. No direct involvement of pituitary body.

*Diagnosis* Tumor of brain (astrocytoma) involving area lying between pineal and pituitary bodies with pressure atrophy of the pituitary body.

Hospital number—248006 Date of admission—August 27, 1930  
Sex—male

*History* Six or seven years ago patient began having pain in the back of his neck and visual difficulties. The condition has changed but little since then.

*Physical examination* Essentially negative except for moderate obesity. Kahn test of blood was negative.

*Ophthalmological examination* Bitemporal hemianopsia. Optic atrophy of both eyes.

*X-ray examination* X-ray studies of the skull reveal destruction of the posterior clinoid processes and marked injury of the anterior with thinning of the floor of the sella turcica.

*Diagnosis* Pituitary tumor. This patient was given deep x-ray therapy with subjective improvement. Date of discharge—October 16, 1930.

Hospital number—253960 Date of admission—November 24, 1930  
Sex—female

*History* A year ago the patient noticed visual disturbances not relieved by glasses. The vision of the right eye failed rapidly until she was blind in that eye. A few months ago there was blurring and haziness of vision in the left eye. She had no other important symptoms.

*Physical examination* Essentially negative except complete blindness of right eye and irregularity of both pupils. Kahn test of the blood was negative.

*Ophthalmological examination* Marked optic atrophy on right and beginning optic atrophy on left. No hemorrhages.

*X-ray examination* X-ray of the skull showed normal anterior clinoid processes with destruction of the posterior and thinning of the floor of the sella turcica.

*Operation* On December 30, 1930 a large pituitary tumor was removed. The patient died January 3, 1931.

*Pathological report* Chromophobe adenoma which replaced a large part of the anterior lobe of the pituitary gland.

*Diagnosis* Tumor of the pituitary gland.

Hospital number—254363 Date of admission—December 2, 1930  
Sex—female

*History* Patient entered the hospital because of blindness of right eye of about one month's duration, and severe headaches. She had had severe headaches over a period of ten years without other complaints until about one year ago. Since then she has had dyspnea, progressive weakness and dimness of vision. One month ago she suddenly became blind in the right eye.

*Physical examination* The physical examination revealed the standard signs of chronic nephritis with hypertension.

*Ophthalmological examination* There was a hemorrhagic retinitis of both eyes. Fundus examination showed choked disc for both eyes—4 diopters. Fields could not be obtained for the right eye but there was marked evidence of optic atrophy of the left with great restriction of the visual field on this side. Kahn test of the blood was negative.

*X-ray examination* Detailed studies of the sella turcica demonstrate a destruction of the floor of the sella turcica and of portions of both the anterior and posterior clinoid processes. Operation was advised against because of the nephritis. The patient was given deep x-ray therapy. Date of discharge March 6, 1931.

*Diagnosis* Chronic nephritis with hypertension. Tumor of the pituitary gland.

Hospital number—257784 Date of admission—January 31, 1931  
Sex—male

*History* Four years before admission patient began to notice weakness and fatigueability. His physician made a diagnosis of diabetes mellitus. His symptoms were relieved by diet until the past two years, when his symptoms have increased so that he could not work, and he sought relief at the hospital. During the past ten years his wife has noticed a marked alteration in his appearance and temperament. He has changed from an aggressive, interested husband into an irritable and careless one. He himself has noticed a thickening of the lower lip and a thickening and widening of the fingers. He has noticed no visual disturbances.

*Physical examination* Ptosis of left upper eyelid. Thick, drooping lip. Tongue strikingly broad and thick. Huge broad hands with thick bulbous fingers. There was a persistent glycosuria of the diabetic type, which was controllable. The glucose tolerance curve was of the diabetic type. Kahn test of the blood was negative.

*Ophthalmological examination* The visual fields showed definite right homonymous hemianopsia.

*X-ray examination* X-ray studies of the skull showed almost complete destruction of the posterior clinoidal processes and apparent thinning of the floor of the sella turcica. X-ray examination of hands showed the typical changes of acromegaly.



*Operation* A pituitary tumor was removed March 26, 1931 The patient died the following day

*Pathological report* Adenoma of the pituitary gland

*Diagnosis* Acromegaly, diabetes mellitus and tumor of the pituitary gland.

# STIMULATION OF GASTRIC PEPSIN BY HISTAMINE

By W SCOTT POLLAND

*(From the Department of Medicine, Stanford University School of Medicine, San Francisco)*

(Received for publication December 2, 1931)

In studying gastric secretion, as many data as possible should be obtained. Although the determination of the enzyme secretory power of the stomach is not as important as the determination of the acid secretory power, it has been pointed out by Polland and Bloomfield (1) that in certain cases the measurement of pepsin output may be a more delicate index of gastric damage than the measurement of acid values. Since the histamine test meal has come into use, it has been generally assumed that the enzyme secretion of the gastric juice is stimulated by histamine. However, recent observations by Gilman and Cowgill (2), and independently by Babkin (3) on pepsin secretion after histamine stimulation indicate that histamine does not stimulate pepsin secretion. Using dogs with Pavlov or Heidenhain pouches, it was found that with the increased volume of secretion in response to histamine there was a relative decrease in pepsin concentration, with the result that the total output of pepsin remained constant. They also noted that after the first stimulation by histamine, the increase in volume of juice was proportionately greater than the decrease in concentration of pepsin, with the result that the total amount of pepsin secreted per unit of time was substantially increased. This effect was interpreted as due to a mechanical washing out of pepsin from the lumina of the glands by the sudden secretion of gastric juice.

Polland and Bloomfield (1) described the curves of pepsin concentration and of total pepsin output before and after stimulation by histamine in normal human subjects, and in people with various gastric lesions. It was observed that after histamine stimulation the concentration of pepsin falls markedly, but the total output was usually increased. It was during the course of these studies that an occasional observation was made on pepsin secretion after repeated histamine stimulation. The results indicated that after each histamine stimulation the pepsin secretion was increased. It was thought that more careful observations were warranted, and the following studies were made.

## MATERIAL AND METHODS

Four male patients were studied. Three presented no evidence of organic disease of the stomach, one was a case of duodenal ulcer. Table 1

shows their age, clinical diagnosis and highest acid and volume response to histamine. Case 1 had high acids and high volumes, case 2 had high acids and normal volumes, case 3 had normal acids and high volumes, and case 4 had low acids and low volumes. This gives a wide range of types of gastric secretion as seen in human subjects.

TABLE I  
*Data concerning cases studied*

Name	Age	Diagnosis	Maximum 10 minute volume of secretion	Maximum free HCl	Maximum total acid
	years		cc	cc N/10 per 100 cc	cc N/10 per 100 cc
Oh	68	Duodenal ulcer	36	140	144
Cl	41	Psychoneurosis	26	136	142
Zi	46	Indigestion	39	102	112
To	49	Irritable colon	8.5	45	68

All subjects were in bed in the hospital and had fasted for twenty-four hours. A mercury-weighted tube was inserted into the stomach and the fasting contents withdrawn. The total secretion over ten-minute periods after an injection of histamine (0.1 mgm per 10 kilos body weight) was then collected exactly as previously outlined (4). After the volumes had returned to the fasting level, a second and later a third injection of the same dose of histamine was given. Volume, titratable acidity and pepsin were measured in all. Titratable acidity was determined with dimethyl and phenolphthalein and pepsin by the method of Pollard and Bloomfield (5). Pepsin is expressed in milligrams of edestin digested by one cubic centimeter of gastric juice in 30 minutes at 37.6 degrees centigrade.

Although the gastric juice was obtained by stomach tube, quantitative technique under these conditions can be simply obtained if certain precautions are followed. First, a cooperative patient, preferably of a phlegmatic temperament must be selected, second, the patient is urged not to swallow saliva and this point is emphasized throughout the test, and third, continuous aspiration must be maintained. Under these conditions a watery clear fluid containing a minimum of mucus is obtained. Duodenal regurgitation rarely occurs, and if bile appears the test is always discarded. This procedure has the distinct advantage that the secretion from the whole stomach is obtained, as contrasted to the partial secretion obtained by pouch methods in dogs. Furthermore, the volume of secretion is large and slight errors in technique are not appreciable.

Table 2 is a complete protocol of Case 2 to illustrate the procedure

TABLE 2  
*Complete protocol of a typical experiment (Case 2)*

Num ber of spec imen	Time	Amount	Character	Acid titratable		Pepsin	
				Free	Total	Edestin digested by 1 cc. of juice	Total edestin digested per 10 minute period
	<i>p.m.</i>	<i>cc.</i>		<i>cc. N/10 H per 100 cc.</i>	<i>cc. N/10 H per 100 cc.</i>	<i>mgm.</i>	<i>mgm.</i>
1	1.00	11.5	Fasting contents Water clear small amount mucus	28	48	2505	28 808
			Histamine 0.6 mgm				
2	1.10	19.5	Water clear	56	74	2505	48 848
3	1.20	24.5	Water clear	120	126	2250	55 125
4	1.30	24.5	Water clear	126	143	1509	36 971
5	1.40	23.5	Water clear	130	136	1640	38 540
6	1.50	19.0	Water clear	136	142	1411	26 809
7	2.00	13.0	Water clear	118	126	1411	18,343
			Histamine 0.6				
8	2.10	17.0	Water clear	122	128	1480	25 160
9	2.20	27.0	Water clear	130	136	1334	36 018
10	2.30	25.0	Water clear	130	136	1200	30 000
11	2.40	25.0	Water clear	126	132	1480	37,000
12	2.50	17.0	Water clear	130	136	1334	22 678
13	3.00	9.0	Water clear	128	134	1270	11 430
			Histamine 0.6				
14	3.10	16.0	Water clear	122	130	1553	24 848
15	3.20	25.0	Water clear	132	138	1480	37 000
16	3.30	26.0	Water clear	126	132	1334	34 684
17	3.40	16.0	Water clear	112	118	1270	20 320
18	3.50	12.5	Water clear	130	136	1005	12,563
19	4.00	6.5	Water clear	124	132	899	5 844

## RESULTS

*Volume and acidity* As has been reported from all previous histamine studies, the volume and acidity rapidly increase, reaching the maximum in 20-30 minutes after injection. The volumes then diminish, while acidity, on the other hand, reaches a level which is maintained throughout the test. The most striking feature of the acid and volume responses was the constancy of their response to repeated doses of the same amount of histamine in a given individual. This is well illustrated in Table 2, the maximum volumes being 24.5 cc., 27 cc., and 26 cc., the maximum total acidities being 142, 136 and 138. This is strong evidence as to the reliability of the technique.

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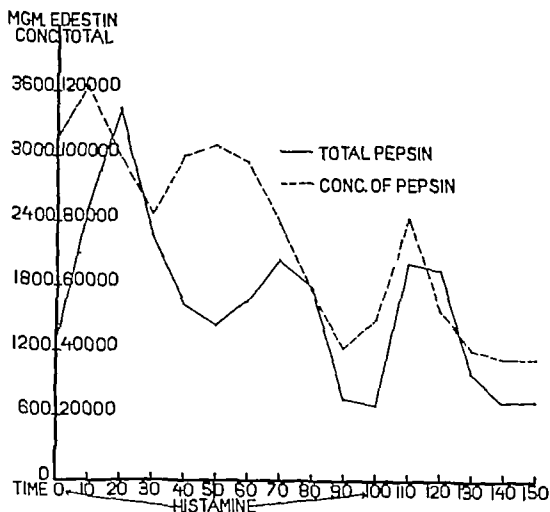


CHART 3 CURVES OF CONCENTRATION, AND TOTAL OUTPUT OF PEPSIN FROM CASE 3 AT TEN MINUTE INTERVALS AFTER REPEATED DOSES OF HISTAMINE

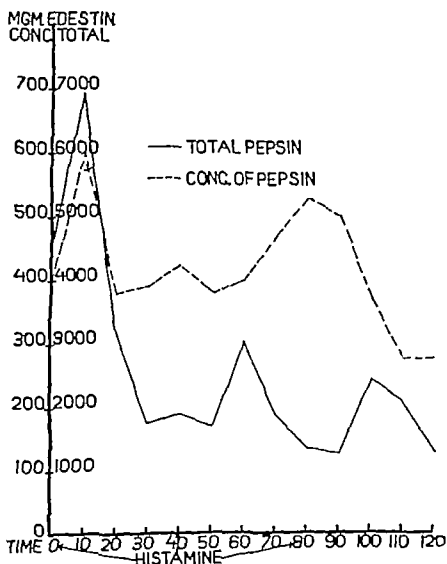


CHART 4 CURVES OF CONCENTRATION, AND TOTAL OUTPUT OF PEPSIN FROM CASE 4 AT TEN MINUTE INTERVALS AFTER REPEATED DOSES OF HISTAMINE

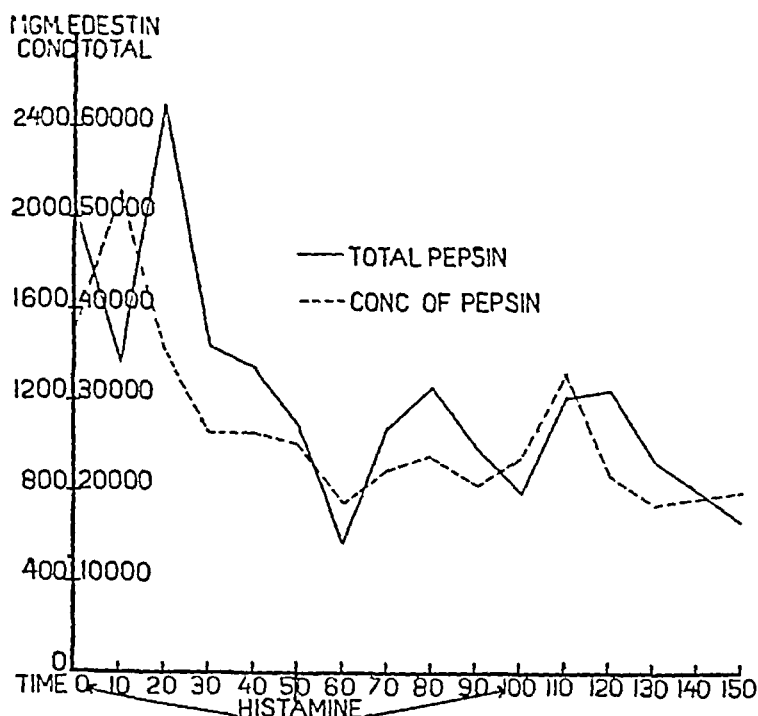


CHART 1 CURVES OF CONCENTRATION, AND TOTAL OUTPUT OF PEPSIN FROM CASE 1 AT TEN-MINUTE INTERVALS AFTER REPEATED DOSES OF HISTAMINE

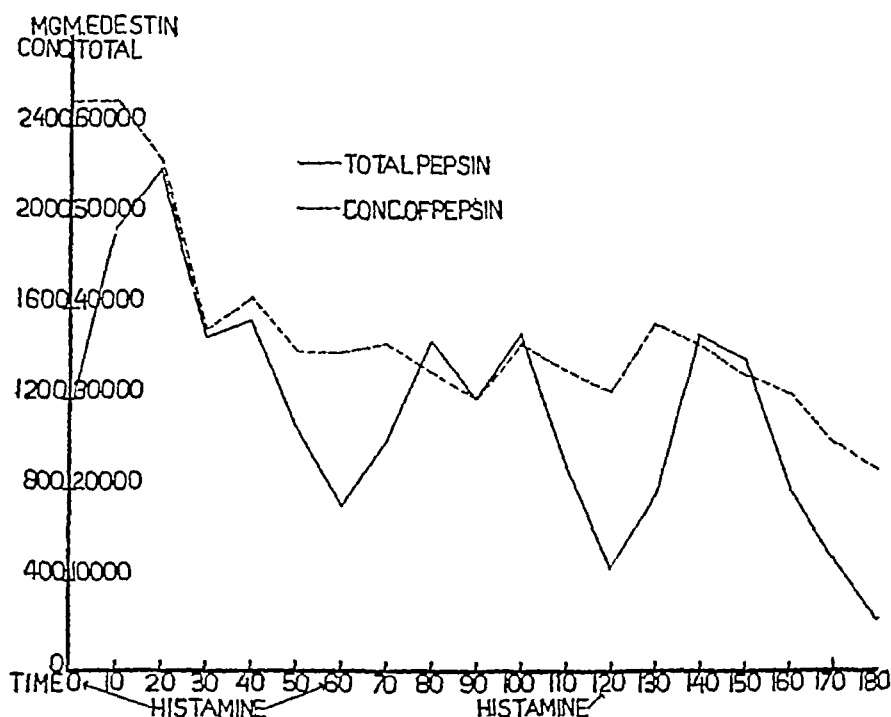


CHART 2 CURVES OF CONCENTRATION, AND TOTAL OUTPUT OF PEPSIN FROM CASE 2 AT TEN-MINUTE INTERVALS AFTER REPEATED DOSES OF HISTAMINE

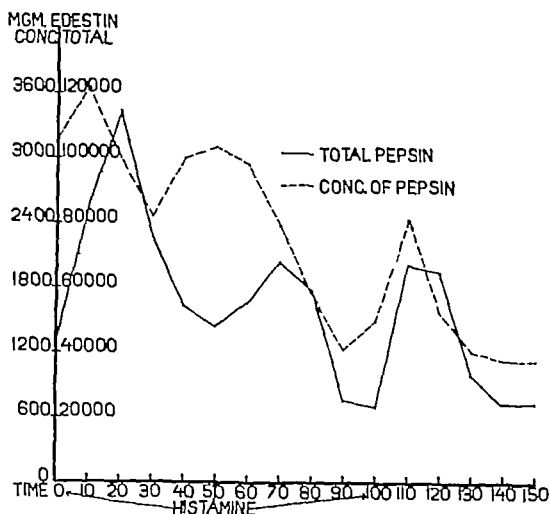


CHART 3 CURVES OF CONCENTRATION, AND TOTAL OUTPUT OF PEPSIN FROM CASE 3 AT TEN MINUTE INTERVALS AFTER REPEATED DOSES OF HISTAMINE

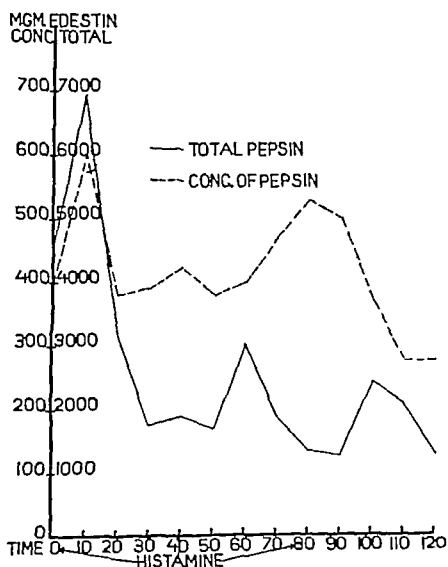


CHART 4 CURVES OF CONCENTRATION, AND TOTAL OUTPUT OF PEPSIN FROM CASE 4 AT TEN MINUTE INTERVALS AFTER REPEATED DOSES OF HISTAMINE



*Pepsin* Charts 1, 2, 3 and 4 show the curves of pepsin concentration and of total output of pepsin per ten-minute period after repeated stimulation in the four cases studied. The total output after the initial dose is striking, and is much higher than the subsequent responses. However, after each stimulus *there is a definite rise in the total output amounting to approximately 100 per cent*, and the magnitude of this rise is practically identical after the second and third injections of histamine. The concentration of pepsin is very variable, and is apparently independent of volume or acid secretion. However, in all cases there is a constant fall in the concentration of pepsin during the height of secretion after the first stimulus.

### DISCUSSION

Contrary to the experience of Gilman and Cowgill, and of Babkin, the results indicate that histamine has a definite stimulating effect upon pepsin. The character of the curve and the similarity of the response after the second and third injection of histamine can only be interpreted satisfactorily by assuming that histamine stimulates the peptic cells. The effect after the first stimulus is probably best explained by a mechanical lavaging of pepsin which has accumulated in the furrows and tubules of the gastric mucosa, as has been pointed out by Gilman and Cowgill, and by Babkin, plus an actual stimulating effect by the histamine. Therefore, it appears that in studying gastric secretion in human subjects, histamine is suitable for determining the capabilities of the pepsin-secreting glands, as well as the acid-secreting glands. Although the two processes are independent, they are influenced by the same stimulus.

### SUMMARY

Evidence is presented to show that in human subjects, histamine stimulates secretion of gastric pepsin.

### BIBLIOGRAPHY

- 1 Polland, W S, and Bloomfield, A L, J Clin Invest, 1930, ix, 107 The Diagnostic Value of Determinations of Pepsin in Gastric Juice
- 2 Gilman, A, and Cowgill, G R, Am J Physiol, 1931, xcvi, 124 The Effect of Histamine Upon the Secretion of Gastric Pepsin
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- 5 Polland, W S, and Bloomfield, A L, J Clin Invest, 1929, vii, 45 A Quantitative Method for the Estimation of Pepsin

# CHEMICAL STUDIES OF THE BLOOD IN HIGH INTESTINAL OBSTRUCTION<sup>1</sup>

## I THE DISTRIBUTION OF PHOSPHORUS AND INTRACELLULAR CHANGES

BY GEORGE MARTIN GUEST AND WILLIAM DEWITT ANDRUS

*(From the Children's Hospital Research Foundation and the Departments of Pediatrics and of Surgery, College of Medicine University of Cincinnati, Cincinnati)*

(Received for publication October 22, 1931)

This paper presents an investigation of the effects of experimental high intestinal obstruction in dogs, with particular emphasis on changes observable in the distribution of phosphorus in the blood. The experiments were undertaken as a part of a series of studies of phosphorus distribution in the blood of man and experimental animals in a variety of pathologic conditions. In dogs, marked changes of the distribution of phosphorus were observed following pyloric and mid-duodenal obstruction, an attempt is here made to correlate these changes with the variations in the blood electrolytes commonly observed in intestinal obstruction.

That experimental high intestinal obstruction brings about or is accompanied by marked changes in the blood has been demonstrated in many investigative studies of this problem reported during the past few years. The more important of these changes are (1) Concentration of the blood indicated by an increased relative cell volume, cell count and serum protein content. (2) Increased nonprotein nitrogen, mostly urea. (3) Low chloride, generally conceded to be due to losses of Cl by secretion into the stomach or the obstructed gut, whence it is vomited or fails to be reabsorbed. (4) High CO<sub>2</sub> content, increased in compensation for the loss of chloride except when there is an accumulation of acids (such as lactic), or when there is great loss of base as well as chloride in the vomitus. It was first pointed out by Gamble and Ross (1925) that the reduction of the ionic content of the body fluids by the losses of electrolytes (base as well as chloride) is the significant factor in the rapid general dehydration of the body that follows pyloric obstruction.

Reviews of the many investigations of intestinal obstruction may be found in the articles by Ellis (1922), Gatch, Trusler and Ayers (1927), Cooper (1928), McVicar and Weir (1929) and in three papers of a sym-

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<sup>1</sup> Presented at a meeting of the Central Society for Clinical Research, Chicago, November 21, 1930

*Pepsin.* Charts 1, 2, 3 and 4 show the curves of pepsin concentration and of total output of pepsin per ten-minute period after repeated stimulation in the four cases studied. The total output after the initial dose is striking, and is much higher than the subsequent responses. However, after each stimulus *there is a definite rise in the total output amounting to approximately 100 per cent*, and the magnitude of this rise is practically identical after the second and third injections of histamine. The concentration of pepsin is very variable, and is apparently independent of volume or acid secretion. However, in all cases there is a constant fall in the concentration of pepsin during the height of secretion after the first stimulus.

### DISCUSSION

Contrary to the experience of Gilman and Cowgill, and of Babkin, the results indicate that histamine has a definite stimulating effect upon pepsin. The character of the curve and the similarity of the response after the second and third injection of histamine can only be interpreted satisfactorily by assuming that histamine stimulates the peptic cells. The effect after the first stimulus is probably best explained by a mechanical lavaging of pepsin which has accumulated in the furrows and tubules of the gastric mucosa, as has been pointed out by Gilman and Cowgill, and by Babkin, plus an actual stimulating effect by the histamine. Therefore, it appears that in studying gastric secretion in human subjects, histamine is suitable for determining the capabilities of the pepsin-secreting glands, as well as the acid-secreting glands. Although the two processes are independent, they are influenced by the same stimulus.

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blood and plasma. Objections may be offered to either method. Long centrifuging is necessary to pack the cells completely (usually more than 30 minutes at 3500 rotations per minute) and even with the greatest care it is difficult to be sure that all the serum has been squeezed from between the cells. Accurate measurement of samples of the packed cells by pipette is difficult. During long centrifuging chemical changes may occur coincident with glycolysis—lactic acid formation, hydrolysis of organic phosphorus compounds, etc.,—which may lead to errors in some of the determinations. Hydrolysis of the phosphoric esters is considerably accelerated in blood samples from animals suffering such types of "intoxication" as those dealt with here, it is, therefore, necessary to make the determinations of the organic phosphorus fractions as quickly as possible after the blood is drawn. Against the indirect method of determining the cell constituents has been the lack of an accurate method for measurement of the relative volume of cells in the blood, but the accuracy of this measurement is considerably increased by the method used in this study. In control studies the indirect method gave closer checks in duplicate determinations of the cell contents than did the direct method. The values for the cells have been calculated by means of the formula

$$\text{mM}_{\text{cells}} = \frac{\text{mM}_{\text{whole blood}} - (\text{mM}_{\text{plasma}} \times \text{Volumes per cent}_{\text{plasma}})}{\text{Volumes per cent}_{\text{cells}}} \times 100$$

The numerical expression of the values for the cell constituents presents a problem somewhat different from that dealt with in the expression of similar values for the constituents of plasma or serum. When these chemical values are expressed in terms of units per volume (mgm per 100 cc or mM per liter) such expressions may lead to confusion if changes in size of the cells reduce or increase considerably the number of cells which can be packed by centrifuging into a given volume. Mere shift of water due to osmotic changes in either cells or plasma may considerably change the figure for a given cell constituent in terms of units per volume, although the actual amount of that substance in the individual cell or its value in the whole blood remains unchanged. A shrinkage of the relative cell volume from 45 per cent to 42 per cent, without change in the number of cells per c.mm., will change the figure for hemoglobin in the packed cells from 35.5 grams per cent to 38.1 grams per cent—granted, of course, that in the meantime the hemoglobin is not actually altered.

These considerations have led to the use of two ratios which give a measure of absolute changes (or constancy in some cases) within the cells which are independent of volume, size of cell, water loss and the like. The ratio, grams of hemoglobin in the whole blood divided by the red cell count in millions per c.mm., is designated "Hb RBC count ratio" and represents the hemoglobin in grams  $\times 10^{-6}$  per million red blood cells. The ratio, mgm of organic acid soluble P in the whole blood divided by the red cell count, is designated "Ester P RBC count ratio" and represents this phosphorus fraction in milligrams  $\times 10^{-4}$  per million red blood cells.

Heparin was used as the anticoagulant for determining the relative cell volume, while potassium oxalate was used in the blood taken for chemical measurements. Oxalate causes some shrinkage of the cells, but it was here employed for the reasons that follow. Heparin contains some P and is, therefore, unsuitable for the P distribution determinations. Since a large number of analyses were done on each blood sample, economy of blood was necessary. The yield of serum from blood simply allowed to clot was so small in the con-

posium on the subject by Foster (1928), Orr and Haden (1928), and McIver and Gamble (1928)

In the present study most attention is directed to the organic acid-soluble phosphorus of the blood, designated by Kay and Byrom (1927) "Ester-P". The nature of the compounds determined in this fraction is not well known, but they are generally believed to be mainly hexose- and glycerophosphoric acid esters (Goodwin and Robison (1924)). In a review of the literature on the distribution of phosphorus in the blood, Peters and Van Slyke (1931) have discussed the nature of the phosphoric acid esters and, pertinent to the problems to be discussed here, the rôle these compounds may play as non-diffusible anions in the cells. They state that part of the alkali in the cells is certainly neutralized by the organic compounds of phosphoric acid, and that there are approximately 30 mM of organic phosphate present in the erythrocytes, also, that the available data indicate that the known phosphoric acid compounds of the cells bind about one equivalent of alkali per mol of phosphoric acid. Although the organic phosphorus compounds of the cells are known to be considerably altered (decreased as well as increased) in several different pathologic conditions, the significance of such changes for the acid-base equilibrium of the blood has not apparently been investigated to any considerable extent.

Many complete determinations of the electrolytes in the blood plasma or serum of man and experimental animals in normal and in various pathologic states have been reported, but few figures are available from which one may visualize the chemical structure of the cells in a manner at all comparable to that of the plasma. In the previous chemical studies of the blood in intestinal obstruction, attention has been directed almost exclusively to changes observed in the plasma, but Haden and Orr (1926) and White and Bridge (1927) demonstrated losses of chloride from various body tissues comparable to the losses of chloride from the blood. Hastings, Murray and Murray (1921) reported the results of three experiments in which they determined the Na, K, Mg, Cl and P in both serum and cells of the blood of dogs before and after pyloric obstruction. In those experiments they found losses of chloride from the blood cells parallel to the losses from the serum, and also slight increases of organic as well as inorganic phosphorus in both serum and cells. Except for the latter study, it appears that only the inorganic phosphates of the serum have been considered in relation to the acid-base equilibrium of the blood in intestinal obstruction.

#### DISCUSSION OF THE METHODS OF STUDY

In attempting to determine the chemical constituents of the blood cells there is a choice of direct analysis of the centrifuged cells or indirect calculation from measurements of the relative volume of the cells and analyses of the whole

*nonprotein nitrogen* The precipitation method of Folin (1930) was used for obtaining the filtrates without hemolysis of cells. Nonprotein nitrogen was determined by the method of Folin Wu (1919), sugar by the method of Folin Wu, as revised by Folin (1929). *Chloride* was determined by an unpublished method of Fiske, described briefly by Fiske and Sokhey (1925). *CO<sub>2</sub> content* was determined by the method of Van Slyke and Neill (1924), using the factors of Van Slyke and Sendroy (1927). *Phosphorus* The method of Fiske and Subbarow (1925) was used for the determination of the inorganic, total acid soluble and total P. For lipin P, the extraction method of Bloor (1918) was used, the P content of an aliquot of the alcohol-ether extract being determined after acid digestion by the Fiske Subbarow method.

#### EXPERIMENTAL

Mongrel dogs of a variety of breeds were used. Blood samples were taken in most instances one or two days before operation, but in a few cases only an hour or so before. The obstructions were made in the mid-duodenum, just below the pancreatic and bile ducts, or at the pylorus. The duodenum or pylorus was divided and both stumps inverted with a double line of sutures. Beginning 12 to 24 hours after operation, in most cases the dogs were allowed water ad libitum to increase the vomiting. Without treatment they lived from 40 hours to five days, the majority dying at from 50 to 72 hours after operation. Dogs with mid-duodenal obstruction seemed to suffer a less rapid fall of the blood chlorides than those with pyloric obstruction, but otherwise there seemed to be little difference in the effects of the two operative procedures. Given salt solution parenterally, the obstructed dogs lived 12 to 20 days and appeared to be in good condition when they were sacrificed—except three that developed signs of distemper and were sacrificed early. Control experiments included the administration of glucose solution parenterally to dogs with pyloric obstruction (see Figure 4), and simple deprivation of food and water in dogs without operation (see Table 4 and Figure 5). The experiments selected for presentation here are typical and have all been repeated, some of them many times.

#### *Phosphorus distribution in the blood of normal dogs (Table 1)*

In Table 1 are given the averages of the values for the distribution of phosphorus determined in the blood of 30 apparently normal dogs. The actual determinations are of the fractions designated (1) inorganic, (2) total acid soluble, (4) total, and (6) alcohol-ether soluble. The values for (3) organic acid soluble 'Ester P,' (5) acid insoluble and (7) non lipin, acid insoluble 'Residual P,' are respectively obtained by the differences 2-1, 4-2, and 5-6. The negative value of the (7) "Residual P" in the plasma is obtained so frequently that it may be suspected that the alcohol-ether extraction actually includes some

centrated blood samples taken late in the period of intoxication that the amounts of blood required to furnish sufficient serum thus were almost prohibitive. Defibrination usually caused some escape of ester-P from the cells, this was especially true when dealing with the pathologic blood samples. The values determined in the whole blood are, of course, unaffected by the anticoagulant and the error in the value calculated for the cells should be less than that suspected in the plasma. Since the P distribution and the intracellular changes were the principal objects of this study, the use of oxalate seemed to be justified.

#### METHODS

The dog's blood was taken usually from the femoral artery (sometimes from the left ventricle when the animal was in extremis) into a 50 or 100 cc glass syringe containing paraffin oil. The blood was at once distributed to separate tubes, and treated as indicated below. The amounts of blood varied according to the needs of the number of analyses to be done, but the proportion of added anticoagulants was kept constant.

For the relative cell volume, cell counts and hemoglobin, 1 mgm heparin to 2 cc of blood.

For the whole blood  $\text{CO}_2$ , plasma  $\text{CO}_2$  and plasma Cl, blood was delivered into a centrifuge tube containing dry potassium oxalate under oil (25 mgm to 10 cc of blood). 10 cc of whole blood for the  $\text{CO}_2$  determination was removed immediately by means of a special pipette described elsewhere (Guest (1931)), and after removal of the excess of oil the blood was centrifuged under solid paraffin to obtain plasma for the  $\text{CO}_2$  and Cl determinations.

For the phosphorus partition in whole blood and plasma, the whole blood Cl, sugar and nonprotein nitrogen, 25 to 30 cc of blood were added to 60 mgm of potassium oxalate.

*Relative cell volume* was determined by a capillary tube method which will be described in detail later (Guest and Siler (unpublished)). After centrifugation of the blood at high speed, about 18,000 rotations per minute for 4 minutes, in small capillary tubes, the lengths of the columns of cells and plasma were read by means of a measuring microscope, from the base of the blood column to the top of the red cells, to the top of the white cells and to the top of the column of plasma. The relative volumes of cells designated "Total" and "RBC only" were calculated from these measurements. *Red cell counts* were made using 0.1 cc of heparinized blood diluted in a 50 cc volumetric flask filled with Hayem's solution. After shaking with glass beads, a drop was transferred from the depths of the flask to a counting chamber and the count made as usual. Counts were made in duplicate and usually agreed within less than 100,000/c.mm, closer checks being obtained by this method than by the use of the small hemocytometer pipette. *Erythrocyte size* The red blood cell volume in 1 c mm of blood, divided by the red blood cell count, in millions per c mm, gives the average erythrocyte size in terms of cubic microns. *Hemoglobin* was determined by the CO capacity method of Van Slyke and Hiller (1928), using 0.2 cc of blood. The figures for the CO capacity in terms of volumes per cent were divided by the factor 1.34 to convert them to grams per cent of hemoglobin. The hemoglobin is expressed in three ways in the tables (1) grams per 100 cc of whole blood, (2) grams per 100 cc. of red cells, (3) "Hb RBC count ratio" as described in the discussion of methods. *Serum protein* was determined by the Abbe refractometer, using the formula of Reiss (1903). In the blood samples late in the period of intoxication it is doubtful whether these values are of more than comparative value. *Sugar and*

*nonprotein nitrogen* The precipitation method of Folin (1930) was used for obtaining the filtrates without hemolysis of cells. Nonprotein nitrogen was determined by the method of Folin Wu (1919), sugar by the method of Folin-Wu, as revised by Folin (1929). *Chloride* was determined by an unpublished method of Fiske, described briefly by Fiske and Sokhey (1925). *CO<sub>2</sub> content* was determined by the method of Van Slyke and Neill (1924), using the factors of Van Slyke and Sendroy (1927). *Phosphorus* The method of Fiske and Subbarow (1925) was used for the determination of the inorganic, total acid soluble and total P. For lipin P, the extraction method of Bloor (1918) was used, the P content of an aliquot of the alcohol-ether extract being determined after acid digestion by the Fiske Subbarow method.

#### EXPERIMENTAL

Mongrel dogs of a variety of breeds were used. Blood samples were taken in most instances one or two days before operation, but in a few cases only an hour or so before. The obstructions were made in the mid-duodenum, just below the pancreatic and bile ducts, or at the pylorus. The duodenum or pylorus was divided and both stumps inverted with a double line of sutures. Beginning 12 to 24 hours after operation, in most cases the dogs were allowed water ad libitum to increase the vomiting. Without treatment they lived from 40 hours to five days, the majority dying at from 50 to 72 hours after operation. Dogs with mid-duodenal obstruction seemed to suffer a less rapid fall of the blood chlorides than those with pyloric obstruction, but otherwise there seemed to be little difference in the effects of the two operative procedures. Given salt solution parenterally, the obstructed dogs lived 12 to 20 days and appeared to be in good condition when they were sacrificed—except three that developed signs of distemper and were sacrificed early. Control experiments included the administration of glucose solution parenterally to dogs with pyloric obstruction (see Figure 4), and simple deprivation of food and water in dogs without operation (see Table 4 and Figure 5). The experiments selected for presentation here are typical and have all been repeated, some of them many times.

#### *Phosphorus distribution in the blood of normal dogs (Table 1)*

In Table 1 are given the averages of the values for the distribution of phosphorus determined in the blood of 30 apparently normal dogs. The actual determinations are of the fractions designated (1) inorganic, (2) total acid soluble, (4) total, and (6) alcohol-ether soluble. The values for (3) organic acid soluble "Ester P," (5) acid insoluble and (7) non lipin, acid insoluble "Residual P," are respectively obtained by the differences 2-1, 4-2, and 5-6. The negative value of the (7) "Residual P" in the plasma is obtained so frequently that it may be suspected that the alcohol-ether extraction actually includes some



TABLE 1

*The distribution of phosphorus in dog's blood Averages of the values determined in 30 normal dogs*

		Whole blood	Plasma	Cells
Relative cell volume, total	<i>volumes per cent</i>	49.5*		
RBC only	<i>volumes per cent</i>	48.4		
RBC	<i>millions per c mm</i>	7.325		
Erythrocyte size	<i>cu mu</i>	66.0		
Phosphorus distribution				
1 Inorganic $\text{NaH}_2\text{PO}_4$ , $\text{Na}_2\text{HPO}_4$	<i>mgm per 100 cc</i>	2.85	3.52	2.16
2 Total acid-soluble	<i>mgm per 100 cc</i>	28.23	3.77	53.2
(2-1)=3 Organic acid-soluble "Ester- Phosphorus"	<i>mgm per 100 cc</i>	25.38	0.25	51.0
4 TOTAL	<i>mgm per 100 cc</i>	43.40	15.68	71.7
(4-2)=5 Acid-insoluble	<i>mgm per 100 cc</i>	15.17	11.91	18.5
6 Alcohol-ether soluble lipin, phosphatides	<i>mgm per 100 cc</i>	14.16†	12.2	16.7
(5-6)=7 Non-lipin, acid-insoluble re- sidual undetermined	<i>mgm per 100 cc</i>	1.0	-0.3	1.8
Ester-P RBC count ratio		3.46		

\* In a larger series of 52 dogs the average relative cell volume is lower, 46.4 per cent, but the average erythrocyte size is 66.1 cu. microns

† Lipin phosphorus was determined in only 17 of these bloods

phosphorus of non-lipin nature that is also extracted in the trichloroacetic acid. The differences are so slight, however, that this error is negligible and for practical purposes the "Lipin" and "Acid-insoluble" fractions in the plasma may be considered identical. The larger value (7) in the whole blood is due perhaps mostly to nucleic acid in the leucocytes.

#### *Mid-duodenal obstruction Dog number 121 (Table 2)*

In this table are shown the principal typical changes observable in the blood of a dog with experimental high intestinal obstruction. Increased concentration of the blood is indicated by the increased serum protein, cell volume and cell count. In this animal there was little change in the erythrocyte size, although in similar experiments there is usually observed a slight shrinkage. The nonprotein nitrogen was markedly increased. The plasma Cl fell and  $\text{CO}_2$  content of the plasma rose. The total P of both plasma and cells increased markedly. Quantitatively, the greatest increase of the individual fractions of the total P was in the organic acid-soluble "Ester-P" of the cells. To make this immediately clearer the actual changes in the P distribution are shown in the lower part of the table by the differences between the values determined before and 48 hours after the operation. The next

TABLE 2

*Dog number 121 Mid-duodenal obstruction Allowed water ad lib Weight 19.5 kilos  
Lost 3.0 kilos Died about 60 hours after operation*

		Before operation			48 Hours after operation		
		Whole blood	Plasma	Cells	Whole blood	Plasma	Cells
Blood cells total	<i>volumes per cent</i>	52.9			59.3		
RBC only	<i>volumes per cent</i>	51.5			56.3		
RBC	<i>millions per c.mm</i>	7.80			8.19		
Erythrocyte size	<i>cu mu</i>	66.0			68.7		
Protein	<i>grams per 100 cc</i>		7.7			10.5	
Nonprotein nitrogen	<i>mgm per 100 cc</i>	26.0			133.0		
Sugar	<i>mgm per 100 cc</i>	91.0			155.0		
Chlorides	<i>mM per liter</i>		104.0			66.3	
CO <sub>2</sub> content	<i>mM per liter</i>		24.3			42.6	
Phosphorus distribution							
1 Inorganic	<i>mgm per 100 cc</i>	2.01	2.69	1.40	6.51	9.41	4.5
2 Total acid soluble	<i>mgm per 100 cc</i>	26.7	2.72	48.0	50.0	10.58	77.0
3 Organic acid soluble	<i>mgm per 100 cc</i>	24.69	0.03	46.6	43.49	1.17	72.5
4 TOTAL	<i>mgm per 100 cc</i>	42.1	13.0	68.0	70.5	26.7	100.5
5 Acid insoluble	<i>mgm per 100 cc</i>	15.4	10.28	20.0	20.5	16.12	23.5
6 Lipin	<i>mgm per 100 cc</i>	13.2	9.6	16.4	15.4	16.1	14.9
7 Non lipin, acid in soluble	<i>mgm per 100 cc</i>	2.2	0.68	3.6	5.1	0.02	8.6
Ester P RBC count ratio		3.16			5.31		
Changes in phosphorus, after operation							
1 Inorganic					+ 4.5	+ 6.72	+ 3.1
3 Organic acid-soluble, ester P					+18.8	+ 1.14	+25.9
4 TOTAL					+28.4	+13.7	+32.5
5 Acid insoluble					+ 5.1	+ 5.84	+ 3.5
6 Lipin					+ 2.2	+ 6.5	- 1.5
7 Non lipin acid insoluble					+ 2.9	- 0.66	+ 5.0

greatest change was in the increased inorganic P of the plasma. The changes in the acid insoluble P were different in the plasma and in the cells; the lipin P was increased in the plasma, while the non lipin, residual P was increased in the cells.

*Mid-duodenal obstruction Dog number 60 (Figure 1)*

This figure presents graphically the changes observed in the blood of a dog at 68 and 96 hours after mid-duodenal obstruction. The dog died 97 hours after operation. The principal changes were (1) Non-protein nitrogen, markedly increased; (2) Decrease of the Cl in both plasma and cells, with increase of the CO<sub>2</sub> content in both. The terminal fall in the CO<sub>2</sub>, sometimes much greater than in this dog, is doubtless due to the accumulation of organic acids which is to be expected at this time, especially with a high nonprotein nitrogen value. (3)

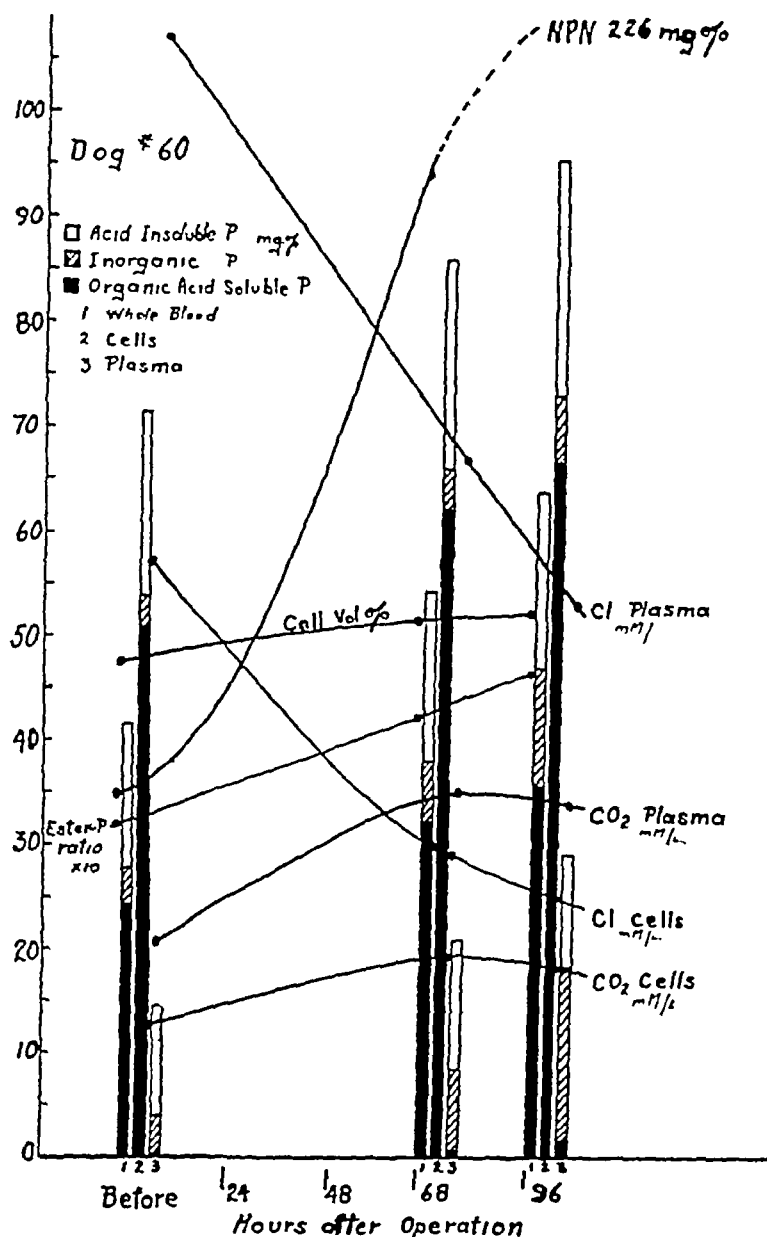


FIG 12 CHANGES IN THE BLOOD OF A DOG, FOLLOWING MID-DUODENAL OBSTRUCTION

\* In these figures the columns 1, 2, 3 represent the total P as mgm per cent in the whole blood, cells and plasma respectively. In the columns the solid, cross hatched and blank portions indicate respectively the organic acid-soluble "Ester-P," the inorganic and the acid-insoluble P. In Figure 4 the lipin P is also represented, at the top of the columns, by perpendicular lines within that portion representing the acid-insoluble P. The curves as drawn represent the chloride and CO<sub>2</sub> contents in terms of millimols per liter (mM/L) and the nonprotein nitrogen as milligrams per 100 cc (mg per cent). The figures for the Ester-P, RBC count ratio, the Hb RBC count ratio and the RBC count in millions per c.mm. have been multiplied by 10 for convenience in making a clearer chart.

**Phosphorus distribution** The ester-P is increased within the cells, and the inorganic P in the plasma. The increases of the acid insoluble P are slight in comparison with these two fractions (4) The serum protein, not shown in the figure, was 7.4 grams per cent before the operation and increased in the two subsequent blood samples after operation to 10.1 grams per cent and 10.6 grams per cent respectively

The changes observed in the blood following pyloric obstruction are similar to those shown occurring after mid-duodenal obstruction in these two experiments. A typical example of pyloric obstruction is displayed in Table 1 of the succeeding paper (Andrus, Guest, Gates and Ashley)

*Mid-duodenal obstruction + salt solution Dog number 78*  
(Figure 2)

The effectiveness of the parenteral administration of salt solution in prolonging life in relieving dehydration and in combatting those changes of the blood Cl, CO<sub>2</sub>, nonprotein nitrogen, etc., which are commonly observed in intestinal obstruction have been demonstrated many times (See the reviews cited above) The two experiments displayed in Figure 2 and Table 3 demonstrate that changes of the blood phosphorus after obstruction are also prevented, at least in great measure, by the administration of salt solution

After mid-duodenal obstruction this dog received daily 40 cc of 0.9 per cent NaCl solution per kilogram body weight, injected subcutaneously. The dog's weight fell progressively from 17.5 kgm to 12.3 kgm on the eleventh day and 12.0 kgm on the sixteenth day after operation. During the first 4 days the blood Cl fell, but thereafter the saline injections brought the Cl in the cells to a normal value and the plasma Cl to 118 mM per liter, considerably above its initial level. The CO<sub>2</sub> during these first 4 days rose, then returned to the initial level. With these changes in the Cl and CO<sub>2</sub>, the organic acid soluble ester-P, after increasing in the cells through the fourth day, returned to normal. In contrast to the cells, the plasma P showed a gradual decrease through the whole period of eleven days. The nonprotein nitrogen progressively decreased. At the sixteenth day, 5 days after the last blood sample was taken, the dog was developing what appeared to be signs of distemper and was sacrificed. The autopsy findings were, however, essentially normal.

*Pyloric obstruction + salt solution treatment interrupted after 18 days*  
Dog number 263 (Table 3)

After pyloric obstruction this dog was given 1000 to 1200 cc. of 0.9 per cent NaCl solution subcutaneously and intraperitoneally daily for 18 days, this represents more than 100 cc per kilogram body weight.

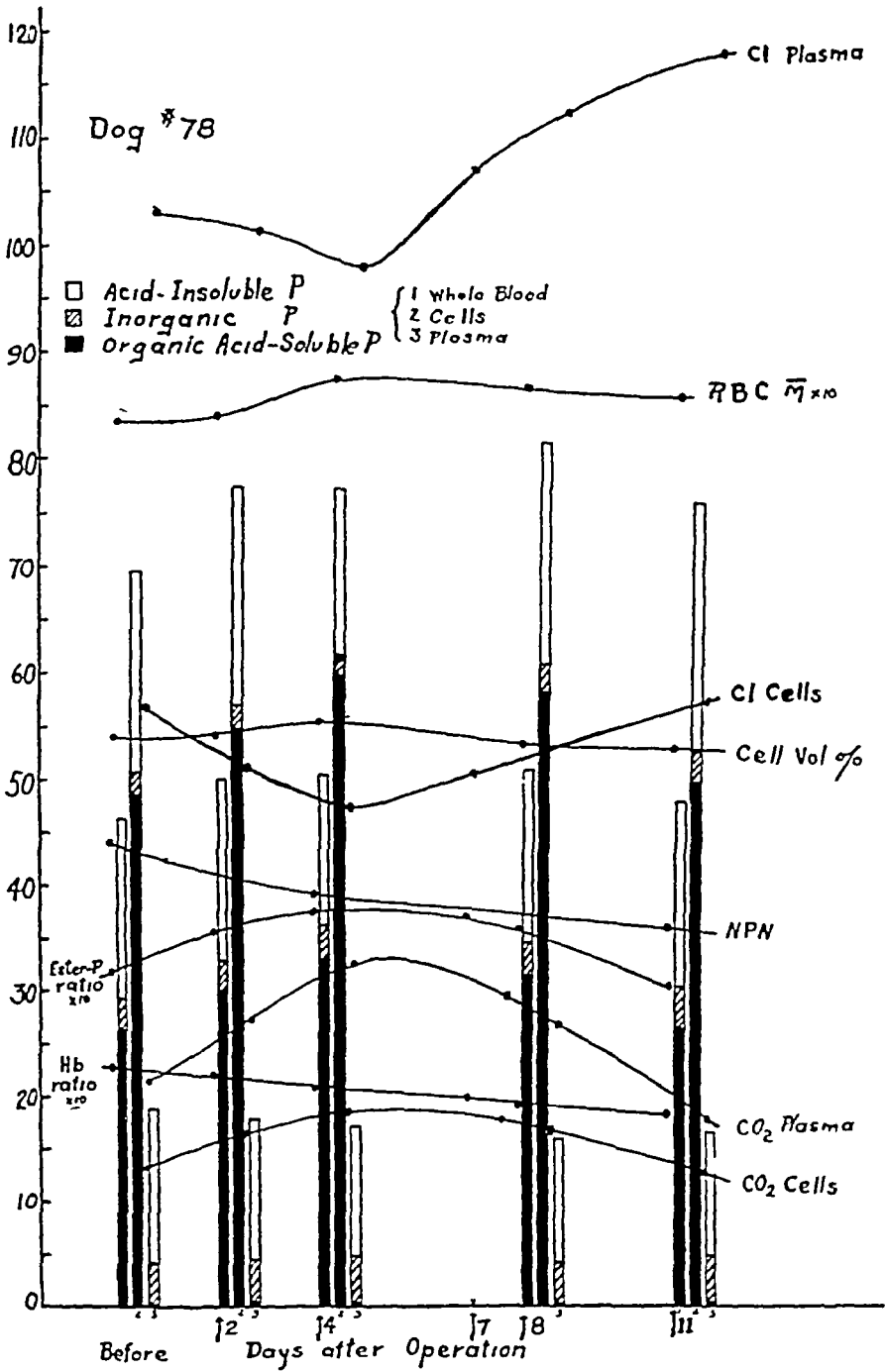


FIG 2 MID-DUODENAL OBSTRUCTION + DAILY SUBCUTANEOUS INJECTIONS OF 0.9 PER CENT NaCl SOLUTION

TABLE 3  
Dog number 263

- 1 Normal
- 2 Pyloric obstruction Received daily subcutaneous injections of 1000-1200 cc. 0.9 per cent NaCl solution for 18 days The dog appeared to be in good condition when the saline injections were stopped
- 3 Last blood sample taken after three days without treatment Death occurred about 4 hours after the blood sample was taken

Days after operation		1	2		3
		Before operation*	Pyloric obstruction		
			Salt solution daily		3 days no salt solution
			11	18	21
Weight	kilos	10.8	9.1	8.0	
Blood cells, total	volumes per cent	47.8	45.4	41.8	47.2
RBC only	volumes per cent	47.3	44.2	40.7	45.8
RBC	millions per c mm	7.00	6.82	6.42	7.91
Erythrocyte size	cu mu	67.5	64.7	63.3	57.9
Hemoglobin, whole blood	grams per 100 cc	16.67	16.32	14.61	17.50
Hemoglobin, cells	grams per 100 cc	35.2	36.9	35.9	38.2
Hb RBC count ratio		2.38	2.39	2.27	2.21
Serum protein	grams per 100 cc.	6.1	5.7	4.9	8.7
Nonprotein nitrogen	mgm per 100 cc	23	19	20	111
Sugar	mgm per 100 cc	91		67	82
CO <sub>2</sub> content plasma	mM per liter	22.0		39.8	50.9
CO <sub>2</sub> content cells	mM per liter	13.2		21.4	26.5
Chloride plasma	mM per liter	110.5	101.0	97.5	65.0
Chloride cells	mM per liter	60.3	56.9	42.5	17.3
Phosphorus distribution					
Whole Blood					
1 Inorganic	mgm per 100 cc	3.65	4.0	3.79	7.96
2 Total acid soluble	mgm per 100 cc	27.0	30.4	30.5	47.8
3 Organic acid soluble	mgm per 100 cc	23.35	25.6	26.7	39.84
4 TOTAL	mgm per 100 cc	43.5	43.7	42.3	67.2
5 Acid insoluble	mgm per 100 cc	16.5	13.3	11.8	19.4
Ester P RBC count ratio		3.33	3.75	4.15	5.03
Plasma					
1 Inorganic	mgm per 100 cc	4.3		4.35	10.46
2 Total acid-soluble	mgm per 100 cc.	4.5		4.4	11.12
3 Organic acid soluble	mgm per 100 cc	0.2		0.05	0.66
4 TOTAL	mgm per 100 cc	16.5	13.5	12.3	25.0
5 Acid insoluble	mgm per 100 cc	12.0		8.0	13.9
Cells					
1 Inorganic	mgm per 100 cc	2.94		3.0	5.16
2 Total acid soluble	mgm per 100 cc.	51.6		66.8	88.8
3 Organic acid-soluble	mgm per 100 cc	48.6	56.0	63.8	83.6
4 TOTAL	mgm per 100 cc	73.0	80.0	84.0	114.4
5 Acid insoluble	mgm per 100 cc	21.4		17.2	25.6

\* Figures in this column are assembled from 3 separate preoperative blood samples

During these 18 days the plasma Cl fell from 110.5 to 97.5 mM, and the Cl of the cells fell from 60.3 to 42.5 mM, indicating that the salt given was not adequate to keep the blood chlorides at their initial level. The dog, however, appeared to be in good condition, and when the saline injections were stopped on the 18th day after operation the blood nonprotein nitrogen was not increased. A blood sample was taken 3 days after the last injection of salt solution and the dog died about 4 hours after this sample was taken. The nonprotein nitrogen in this last blood sample was 111 mgm per 100 cc. The chlorides had decreased to 65.0 mM in the plasma and to 17.3 mM in the cells, while the CO<sub>2</sub> was increased in both plasma and cells. During the first 18 days the plasma total P diminished, in the cells the organic acid-soluble "Ester-P" fraction increased slowly from 48.6 to 63.8 mgm per 100 cc. After the abrupt interruption of the saline injections the ester-P in the cells rose in 3 days to 83.6 mgm per 100 cc, while the total P of the cells reached 114.4 mgm per 100 cc. In the first 18 days the red blood cell count fell somewhat, but rose in the last 3 days. The serum protein had fallen to 4.9 grams per cent on the 18th day, but in the last sample had increased to 8.7 grams per cent. The *Hb RBC count ratio* changed very little during this whole period, an indication that in this important characteristic the red cells had not changed. Contrast with this the increasing *Ester-P RBC count ratio*.

*Pyloric obstruction + glucose solution Dog number 298*  
(Figure 3)

It has been argued that the increase of inorganic phosphate in the blood in intestinal obstruction is due to a failure of renal excretion of waste endogenous phosphates (see Discussion). The experiment shown in Figure 3 was performed to see whether the promotion of marked diuresis by the parenteral administration of water in the form of glucose solution would prevent the increase of phosphates in the blood.

After pyloric obstruction this dog received daily injections of 5 per cent glucose solution subcutaneously and intraperitoneally. The animal lived 6½ days, a longer time than any of the dogs lived that did not receive salt solution. The body weight fell from 19.7 to 18.0 kilograms. The blood nonprotein nitrogen increased slowly, if compared with the nonprotein nitrogen increase in the untreated dogs with pyloric obstruction. The erythrocyte size and hemoglobin content of the cells remained remarkably constant, although there was a considerable decrease (6,700,000 to 5,375,000 per c mm) in the red blood cell count. The serum protein was 7.9 grams per cent before operation and increased to 8.3 and 8.5 grams per cent on the fourth and sixth days respectively. By the columns in the figure, representing the P distribution on the third and fifth days after operation, it may be seen that the increase

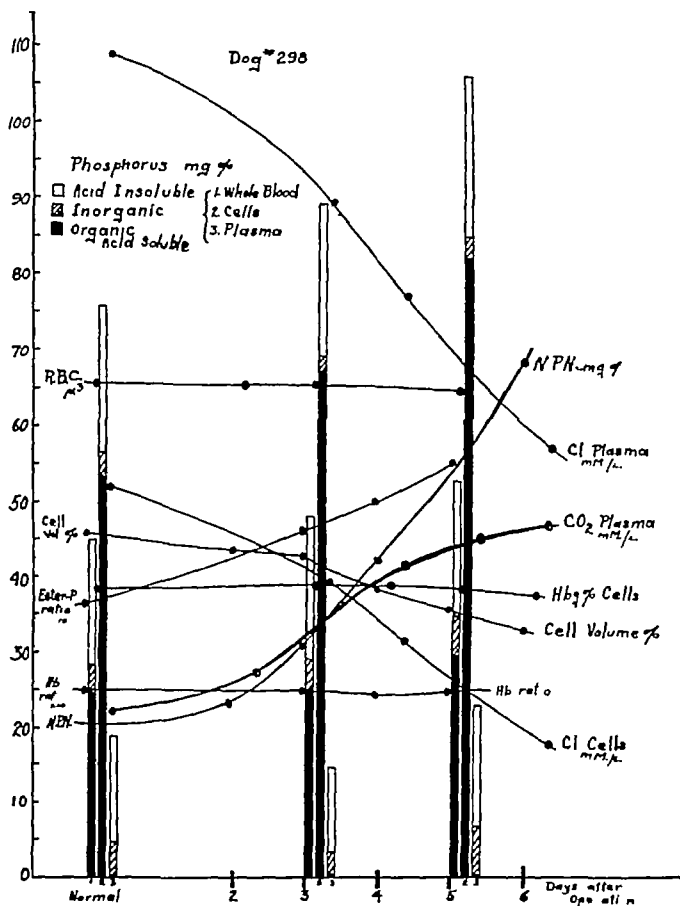


FIG 3 PYLORIC OBSTRUCTIONS + DAILY SUBCUTANEOUS AND INTRAPERITONEAL INJECTIONS OF 5 PER CENT GLUCOSE SOLUTION

of the ester P in the cells, as well as the losses of chloride from both plasma and cells, occurred exactly as observed in the obstructed dogs left without treatment.



*Effects of deprivation of food and water Dog number 69 (Table 4)*  
*Dog number 278 (Figure 4)*

The two control experiments, shown in Table 4 and Figure 4, are cited to show that in normal dogs simple deprivation of food and water even after 16 and 18 days did not bring about any such remarkable changes of the phosphorus distribution in the blood as are observed in the dogs with intestinal obstruction

The dog number 69 (Table 4), deprived of food and water for 16 days, showed rather remarkable constancy in the blood chemical values. Note that the increasing chloride and serum protein indicate a con-

TABLE 4  
*Dog number 69 Deprived of food and water*

		Before	Days of starvation			
			2	6	11	16
Weight	kilos	12.5	12.0	10.5	9.5	8.6
Blood cells, total	volumes per cent	54.0	56.0	55.4	56.9	48.2
RBC	millions per c mm	8.19	8.23	8.80	8.97	8.26
Hemoglobin, whole blood	grams per 100 cc			19.25	18.24	16.94
Hb RBC count ratio				2.18	2.03	2.05
Serum protein	grams per 100 cc	6.40	8.49	7.97	9.54	9.83
Nonprotein nitrogen	mgm per 100 cc	31.0	39	39	30	120
Sugar	mgm per 100 cc	98	100	129	96	129
CO <sub>2</sub> content, plasma	mM per liter	22.48	22.29	21.68	23.87	17.9
CO <sub>2</sub> content, cells	mM per liter	14.4	13.8	14.6	17.0	12.3
Chloride, plasma	mM per liter	107	112.0	119.0	123.0	132.7
Chloride, cells	mM per liter	56.1	64.6	61.4	68.5	69.8
Phosphorus distribution						
Whole blood						
1 Inorganic	mgm per 100 cc	2.29	2.91	3.02	3.45	8.4
2 Total acid-soluble	mgm per 100 cc	26.9	27.6	30.1	30.3	33.3
3 Organic acid-soluble	mgm per 100 cc	24.6	24.7	27.1	26.8	24.9
4 TOTAL	mgm per 100 cc	38.9	42.8	45.4	45.5	49.4
5 Acid-insoluble	mgm per 100 cc	12.0	15.2	15.3	15.2	16.1
Ester-P RBC count ratio		3.00	3.00	3.08	2.98	3.01
Plasma						
1 Inorganic	mgm per 100 cc	2.87	3.81	3.71	4.06	10.4
2 Total acid-soluble	mgm per 100 cc	3.06	4.08	4.00	4.66	10.8
3 Organic acid-soluble	mgm per 100 cc	0.2	0.3	0.3	0.6	0.4
4 TOTAL	mgm per 100 cc	10.15	15.6	15.5	13.5	21.7
5 Acid insoluble	mgm per 100 cc	7.1	11.5	11.5	8.8	10.9
Cells						
1 Inorganic	mgm per 100 cc	1.80	2.20	2.46	2.99	6.25
2 Total acid soluble	mgm per 100 cc	47.2	46.1	51.1	49.7	57.4
3 Organic acid-soluble	mgm per 100 cc	45.4	43.9	48.6	46.7	51.2
4 TOTAL	mgm per 100 cc	63.4	64.2	69.5	69.7	79.1
5 Acid-insoluble	mgm per 100 cc	16.2	18.1	18.4	20.0	21.7

siderable concentration of the blood. In the last sample the non-protein nitrogen is high, and the inorganic P is considerably elevated, but even in this sample the changes in the phosphorus are almost negligible except for the inorganic fraction.

In the dog number 278 (Figure 4), deprived of food and water, there was a considerable decrease in the total phosphorus of the blood cells in the first six days, without any appreciable change in the phosphorus of the plasma. Subsequently there was a slow increase of the total cell phosphorus, but in the whole period of 18 days these changes are practically limited to the organic acid soluble fraction. Note the progressive steady increase of the plasma chloride to 129 mM per liter. On the 18th day the blood nonprotein nitrogen was high, and the dog was found dead on the morning of the 20th day.

#### DISCUSSION

The increases of inorganic phosphates in the blood in intestinal obstruction have been explained by the hypothesis, offered by Atchley and Benedict (1927), that the kidneys fail to excrete the endogenous waste phosphates. Wakeman, Peters and Lee (1931) found that in pyloric obstruction the concentration of P in the urine was very high, but it is perhaps possible that even a partial "retention" might result in the accumulation of phosphates in the blood since under these conditions there is excessive tissue catabolism which makes necessary a greatly increased excretion of waste phosphates. Such failure of excretion might be due to a diminution of kidney function (McQuarrie and Whipple (1919)) associated with the toxic nephritis described by Brown, Eusterman, Hartman and Rowntree (1923) as part of the intoxication that accompanies intestinal obstruction, or it might be due simply to lack of water, the result of dehydration brought about by vomiting. The increase of the nonprotein nitrogen in the blood might be explained on either basis, or both, and the increased nonprotein nitrogen figure may be accepted as an indication of a failure of excretion of various endogenous waste substances. The increase of ester-P in the cells seems not so easily explainable, but here again the diminished excretory function of the kidneys must be considered in view of the work of Kay (1926) and of Eichholtz, Robison and Brull (1925) which indicated that the inorganic phosphates of the urine may be derived from the ester-P of the blood by enzyme hydrolysis of these esters as the blood passes through the kidneys. Thus, failure of renal function might possibly include suppression of such enzymatic processes and, therefore, allow the accumulation of organic as well as inorganic phosphates in the blood. This subject will be further discussed in a later paper in which will be reported studies—similar to those reported here—of experimental nephritis and the effects of bilateral ligation of the ureters in dogs.

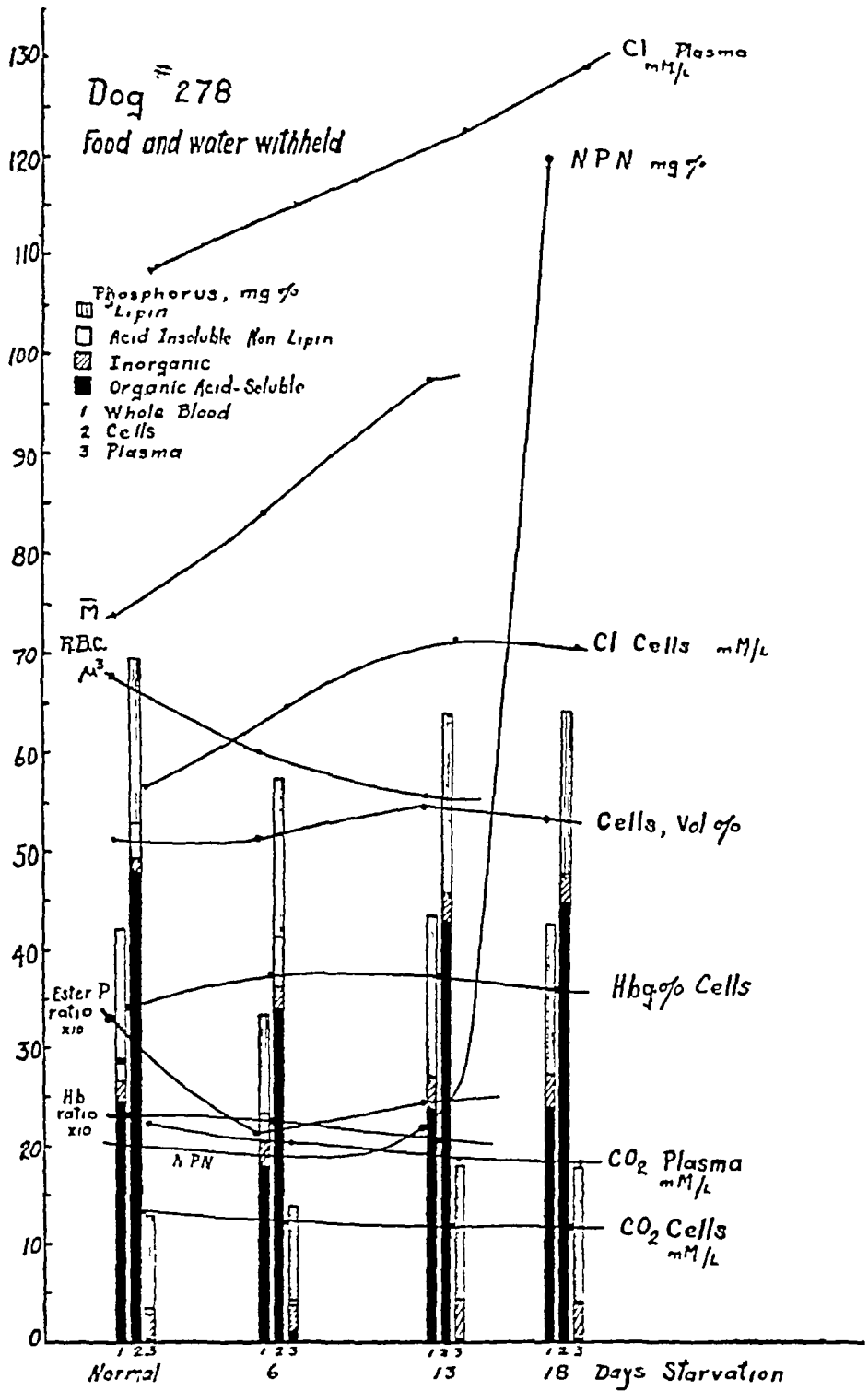


FIG 4 CHANGES IN THE BLOOD OF A DOG DEPRIVED OF FOOD AND WATER

However, even granting that diminished renal function may be partly responsible for an accumulation of phosphates in the blood, the mechanism of the increase of ester-P in the blood cells remains obscure. The phosphorus compounds determined in this fraction are extremely labile, various factors decreasing and increasing their concentration. Haldane, Wigglesworth and Woodrow (1924) and Kay (1924) have demonstrated that chloride acidosis (induced by the feeding of  $\text{NH}_4\text{Cl}$ ) brings about a great reduction of the ester P of the blood cells. The mechanism of this effect has not been completely explained but in view of the evidence cited in the introduction, that the phosphoric esters are bound to alkali in the cells, it seems possible that the excess of  $\text{Cl}'$  ions in such a state of acidosis is capable of displacing the ester P from this alkali. Following this argument, if  $\text{Cl}'$  is lost from the cells the ester P may be retained by the alkali from which  $\text{Cl}'$  was lost much as  $\text{HCO}_3'$  is known to be retained by the alkali of the blood from which Cl is lost. At any rate, in these dogs with intestinal obstruction there is observed greater parallelism between the losses of Cl from the cells and the increases of the ester-P in the cells than between any of the other chemical changes.

In the two experiments shown in Figure 2 and Table 3, the administration of salt solution parenterally prevented an accumulation of the nonprotein nitrogen in the blood and there was no appreciable change in the inorganic P, yet there was an increase of the ester P paralleling closely the losses of Cl from the cells. Unless the excretion of waste phosphates is quite different from the excretion of waste nitrogenous products, it would appear that at least in these experiments the accumulation of phosphorus was not due to failing renal function. In the two dogs deprived of food and water (Table 4 and Figure 4) the terminal increases of the nonprotein nitrogen in the blood before death were not accompanied by any significant increase of the ester P of the blood, even when in one dog (number 69) the inorganic P of the plasma was increased to 10.4 mgm per 100 cc.

#### SUMMARY

Following experimental pyloric and mid-duodenal obstruction in dogs, marked changes in the distribution of phosphorus in the blood have been observed. The phosphorus was partitioned as the following fractions in the whole blood, plasma and cells: Inorganic, acid-soluble, organic acid-soluble or "Ester P", acid insoluble, alcohol-ether soluble or lipin P, total phosphorus. The most important changes were marked increases in the fraction designated "Ester P" which has an average normal value of about 50 mgm per 100 cc. in the cells and only 0.3 mgm per 100 cc. in the plasma. The increases of the ester-P were much greater than the changes in any of the other phosphorus fractions of the cells or plasma.

Changes in chloride and  $\text{CO}_2$  content of both plasma and cells were compared with concomitant changes in the distribution of phosphorus. In all the experiments there was a close correlation between the progressive losses of chloride from the blood cells and the increases of organic acid-soluble phosphorus. It seems likely that as the organic phosphorus compounds increased they were bound to the alkali in the cells from which  $\text{Cl}'$  was lost.

The parenteral administration of  $\text{NaCl}$  solution to obstructed dogs prevented the increases of organic acid-soluble phosphorus in the blood cells to about the same degree that it prevented the losses of chloride from the blood cells.

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# CHEMICAL STUDIES OF THE BLOOD IN HIGH INTESTINAL OBSTRUCTION

## II THE RELATION BETWEEN "TOXEMIA" AND CHEMICAL CHANGES

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In the preceding paper (Guest and Andrus, 1932) certain of the principal changes occurring in the blood of dogs following simple pyloric or mid-duodenal obstruction have been discussed. Experiments were reported in which the distribution of phosphorus in the blood of dogs with obstruction was determined, and changes in various fractions of the blood phosphorus were correlated with other chemical changes of the blood which are generally known to be associated with intestinal obstruction. The greatest change in the blood phosphorus was in that fraction designated as the 'organic acid soluble ester P' practically all of which is contained in the cells. Changes in the chloride content of both plasma and cells were compared with concomitant changes in the distribution of phosphorus, and a close correlation between the progressive losses of chloride from the blood cells and the increases of the ester P of the cells was demonstrated. From these findings it was concluded that in the acid base equilibrium of the cells these organic phosphorus compounds had some sort of a reciprocal relationship to the cell chloride, and that as these phosphorus compounds increased they were bound to the alkali in the cells from which chloride was lost, much as  $\text{HCO}_3'$  is known to be retained by the alkali of the blood from which  $\text{Cl}'$  is lost. In those experiments it was also shown that the parenteral administration of NaCl solution prevented increases of ester P in the cells to about the same degree that it prevented losses of chloride.

The two theories most commonly accepted in explanation of the cause of death in intestinal obstruction are (1) that death is due to dehydration and disturbance of the acid base equilibrium of the body which follows the loss of electrolytes and water by vomiting and by failure of reabsorption of gastro intestinal secretions from the obstructed bowel, (2) that death is due to a state of intoxication of the tissues brought about by toxic substances absorbed from the obstructed bowel.





into dogs cause a marked augmentation of gastric, pancreatic and intestinal secretion. Accordingly it seems probable from this as well as other evidence obtained by other workers, that the fall in blood chlorides in obstruction is due to their accelerated passage into the alimentary tract and failure of reabsorption."

In the experiments here described, histamine is employed as a known substance which is at least closely related to the toxic substances present in and absorbable from the obstructed bowel. Injected subcutaneously, histamine stimulates a rapid flow of gastric juice of high HCl content. In normal animals such injections cause only slight lowering of the blood chlorides, even when given at frequent intervals and continued over a long period (Lim and Ni (1926), Drake and Tisdall (1926)), presumably this is because most of the secreted gastric juice is reabsorbed after passing through the pylorus. However, if the flow of gastric juice thus stimulated is allowed to escape, the repeated injections of histamine are rapidly followed by marked blood chemical changes and death. Lim and Ni reported experiments in which dogs with Pavlov pouches were subjected to hourly subcutaneous injections of histamine in doses of 0.2 mgm per kilogram body weight. One such dog (G 17), given increasing doses of histamine, averaging 1.9 mgm per hour, succumbed at the end of 10 hours, during this time the chloride of the whole blood fell from 308 mgm per 100 cc (87 mM) before the injections to 222 mgm per 100 cc (62.7 mM) at the 10th hour. "Gastric secretion was obtained up till the last, the total secreted being 743 cc of juice and 3190 mgm of Cl in spite of the dehydration and large amount of Cl lost, amounting in one animal to 49 per 100 cc. of the total body Cl, the gastric glands continue to secrete." Similar results were obtained when unoperated animals were injected with repeated doses of histamine while the gastric secretions were continuously aspirated by means of a Rehfuess tube. A gastrectomized dog, on the other hand, showed no fall in the blood Cl after histamine injections. Two points may be considered established by such experiments as these: first, that the secretion of HCl with the gastric juice is the fundamental cause of the fall of the blood chloride when these secretions are lost from the body, secondly, that histamine will markedly accelerate such losses.

Certain objections may here be made to some of the conclusions drawn from previous experimental studies of this problem in which various preparations of filtrates or toxic substances isolated from the contents of the obstructed bowel were injected intravenously to prove their toxicity in normal animals. Such an injection may provoke those symptoms generally known as "shock," and immediate death, but clinically one rarely sees such fulminant progress of symptoms leading to death. Histamine when injected intravenously immedi-

The efficacy of the administration of NaCl solution in prolonging the life of animals with simple experimental high obstruction is one of the strongest indications that in this condition the loss of water and electrolytes is the principal lethal factor, on the other hand, with "closed loops" or in obstruction with strangulation such treatment is less efficacious and in these circumstances the factor of toxemia appears to have greater importance

Clinically, one seldom encounters circumstances capable of producing the picture of simple obstruction as it is experimentally produced in animals, because constrictions and other factors introduce the complications of strangulation in a high percentage of cases. With strangulation of the intestine, death occurs much more quickly than in simple obstruction, and much work has been done to demonstrate that in such cases the greater severity of symptoms is due to toxic substances which appear in the obstructed bowel. Filtrates and certain extracts of the bowel contents from above the point of obstruction are extremely toxic when injected into animals, and many attempts have been made to isolate and identify the substance, or substances, responsible for this toxicity. A review of this work may be found in the article by Cooper (1928). Whipple and his collaborators (1916) believed the toxic substances to be of proteose nature, while Gerard (1922) and others have pointed out the similarity in the pharmacologic effects of these extracts and of histamine. Most of the evidence accumulated from many different studies indicates that the toxic agent is some product of protein degradation, probably several related substances, and that many of the properties and effects of these substances are like those of histamine. Apparently the toxic substance is not absorbed by the normal mucosa (Davis (1914), Murphy and Brooks (1915)) but it has been demonstrated that the mucosa of the bowel above the point of obstruction is not normal, that increased tension within the bowel may cause absorption to take place (Stone and Firor (1924)) and that breaks in the continuity of the mucosa may be produced (Murphy and Brooks (1915), Van Buren (1920)) which allow the toxic substance to enter the tissues.

The presence of toxic substances in the obstructed bowel cannot be denied, but there has been much controversy over their importance in causing death, compared to the importance of the losses of electrolytes and the accompanying chemical changes of the body fluids. The studies to be reported here were undertaken with the idea that these two theories of the cause of death in intestinal obstruction could be better correlated, and that the chemical changes and the "toxemia" could be demonstrated to be interdependent. Dragstedt (1928) has already postulated such a relationship, as follows: "the toxic fractions in obstruction fluids are powerful secretagogues, and when injected

TABLE 1

*Dog number 266 Pyloric obstruction Allowed water ad lib, starting 18 hours after operation Lived 71 hours*

		Normal	Hours after operation	
			48	70
Weight	kgm	23.2	21.2	20.1
Blood cells total	volumes per cent	51.5	56.4	50.8
RBC only	volumes per cent	50.1	53.6	48.9
RBC	millions per c.mm	7.91		7.75
Erythrocyte size	cu mu	63.3		63.1
Hemoglobin whole blood	grams per 100 cc	17.19		17.12
Hemoglobin cells	grams per 100 cc	34.3		35.0
Hb RBC count ratio		2.17		2.21
Serum protein	grams per 100 cc	9.2	10.6	11.0
Nonprotein nitrogen	mgm per 100 cc	30*	100	169
Sugar	mgm per 100 cc	91	163	35.3
CO <sub>2</sub> content plasma	mM per liter	22.0*	32.76	27.44
CO <sub>2</sub> content cells	mM per liter	13.0*	18.84	14.25
Chloride, plasma	mM per liter	106.0*	78.5	73.5
Chloride cells	mM per liter	58.0*	36.8	28.2
Phosphorus distribution				
Whole blood				
1 Inorganic	mgm per 100 cc	3.2	5.26	8.77
2 Total acid soluble	mgm per 100 cc	29.4	42.2	52.2
3 Organic 'Ester P	mgm per 100 cc	26.2	36.94	43.43
4 TOTAL	mgm per 100 cc	49.1	69.0	76.2
5 Acid insoluble	mgm per 100 cc	19.7	26.8	24.0
Ester P RBC count ratio		3.31	4.35†	5.6
Plasma				
1 Inorganic	mgm per 100 cc	3.8	7.47	13.8
2 Total acid soluble	mgm per 100 cc	4.2	8.16	14.8
3 Organic 'Ester P	mgm per 100 cc.	0.4	0.7	1.0
4 TOTAL	mgm per 100 cc.	21.6	29.7	34.4
5 Acid insoluble	mgm per 100 cc	17.4	21.5	19.6
Cells				
1 Inorganic	mgm per 100 cc	2.63	3.55	3.90
2 Total acid soluble	mgm per 100 cc	53.1	68.5	88.4
3 Organic Ester P	mgm per 100 cc	50.5	65.0	84.5
4 TOTAL	mgm per 100 cc	75.0	99.4	116.7
5 Acid insoluble	mgm per 100 cc	21.9	30.9	28.3

\* Assumed normal values taken for comparison

† The RBC count was lost in this second sample. Assuming that the erythrocyte size did not change the RBC count in the second sample would be 8.48 millions per c.mm. The value 4.35 for the ester P ratio is interpolated from this figure.

ately provokes a train of severe symptoms, whereas subcutaneous injections have less severe effects which may be considered more physiologic in nature. The slow absorption of the toxic substances from the bowel, when this does occur, must have effects more nearly like those of subcutaneous injections than like those of a large overwhelming dose suddenly injected intravenously. Moreover, the injection of a large dose of toxic substance, either intravenously or subcutaneously, may be followed by a severe reaction which is not necessarily simply an exaggeration of the effect of a small dose. If one is to reproduce the conditions that are encountered clinically, it would seem that the repeated subcutaneous injections of very small doses of these toxic substances, at short intervals, should more nearly duplicate the conditions imposed by their gradual absorption into the tissues from the obstructed bowel, this method was, therefore, employed in the experiments that follow.

#### EXPERIMENTAL

*Methods* The methods used in the experiments were the same as those described in the preceding paper.

##### *Pyloric obstruction Dog number 266 (Table 1)*

In this table are given figures which illustrate the typical changes observed in the blood of a dog with simple pyloric obstruction. The principal changes demonstrated in this experiment are (1) The relative cell volume increased in the second blood sample but decreased again in the third sample. The erythrocyte size was unchanged in the last sample, but ordinarily there is observed a slight diminution in this value. (2) The hemoglobin content of the cells remained practically unchanged, demonstrating that in this important constituent the red cells remained normal. (3) The serum protein and the nonprotein nitrogen increased. (4) The chloride fell markedly in both plasma and cells. (5) The  $\text{CO}_2$  content of both plasma and cells increased in the second sample, but fell terminally, presumably because of the increase of organic acids which is to be expected at this time. (7) There was a marked increase of the organic acid-soluble ester-P of the cells, evident in the figure for this fraction in the packed cells (84.5 mgm per 100 cc) and in the ratio of Ester-P/RBC count. The next greatest change of the blood phosphorus was in the inorganic fraction in the plasma.

##### *Pyloric fistula + histamine Dog number 262 (Table 2)*

In this dog, under ether anesthesia, the pylorus was severed, the distal stump inverted and sutured and a pyloric fistula created through the abdominal wall. With a soft rubber tube in the fistula opening, the dog was kept in a frame for the collection of the gastric secretions.

TABLE 1

*Dog number 266 Pyloric obstruction Allowed water ad lib starting 18 hours after operation Lived 71 hours*

		Normal	Hours after operation	
			48	70
Weight	kgm	23.2	21.2	20.1
Blood cells total	volumes per cent	51.5	56.4	50.8
RBC only	volumes per cent	50.1	53.6	48.9
RBC	millions per c.mm	7.91		7.75
Erythrocyte size.	cu. mu	63.3		63.1
Hemoglobin whole blood	grams per 100 cc.	17.19		17.12
Hemoglobin cells	grams per 100 cc.	34.3		35.0
Hb RBC count ratio		2.17		2.21
Serum protein	grams per 100 cc	9.2	10.6	11.0
Nonprotein nitrogen	mgm per 100 cc	30*	100	169
Sugar	mgm per 100 cc	91	163	35.3
CO <sub>2</sub> content plasma	mM per liter	22.0*	32.76	27.44
CO <sub>2</sub> content, cells	mM per liter	13.0*	18.84	14.25
Chloride, plasma	mM per liter	106.0*	78.5	73.5
Chloride cells	mM per liter	58.0*	36.8	28.2
Phosphorus distribution				
Whole blood				
1 Inorganic	mgm per 100 cc	3.2	5.26	8.77
2 Total acid soluble	mgm per 100 cc	29.4	42.2	52.2
3 Organic 'Ester P	mgm per 100 cc	26.2	36.94	43.43
4 TOTAL	mgm per 100 cc	49.1	69.0	76.2
5 Acid insoluble	mgm per 100 cc	19.7	26.8	24.0
Ester P RBC count ratio		3.31	4.35†	5.6
Plasma				
1 Inorganic	mgm per 100 cc.	3.8	7.47	13.8
2 Total acid soluble	mgm per 100 cc.	4.2	8.16	14.8
3 Organic 'Ester P	mgm per 100 cc.	0.4	0.7	1.0
4 TOTAL	mgm per 100 cc	21.6	29.7	34.4
5 Acid insoluble	mgm per 100 cc	17.4	21.5	19.6
Cells				
1 Inorganic	mgm per 100 cc	2.63	3.55	3.90
2 Total acid-soluble	mgm per 100 cc	53.1	68.5	88.4
3 Organic 'Ester P	mgm per 100 cc	50.5	65.0	84.5
4 TOTAL	mgm per 100 cc.	75.0	99.4	116.7
5 Acid insoluble	mgm per 100 cc	21.9	30.9	28.3

\* Assumed normal values, taken for comparison

† The RBC count was lost in this second sample. Assuming that the erythrocyte size did not change the RBC count in the second sample would be 8.48 millions per c.mm. The value 4.35 for the ester P ratio is interpolated from this figure.

After apparent recovery from the anesthesia, 3 hours after operation, 1 mgm doses of histamine in 1 cc of 0.9 per cent NaCl solution were injected subcutaneously at half-hourly and hourly intervals. These injections were continued until the dog died at the end of 16 hours (19 hours after the operation) by which time a total of 20 mgm of histamine had been injected. During the first 12 hours of the injections the flow of gastric juice was almost continuous, observed as a small thin mucoid stream which was visibly accelerated by each injection of histamine. After 12 hours, the flow diminished considerably, and the response to each injection of histamine was much less. The total gastric secretions collected contained the equivalent of 1050 cc of N/10 HCl. Complete analyses on the preliminary small blood sample

TABLE 2

*Dog number 262 Pyloric fistula + histamine Died 16 hours after start of hourly subcutaneous injections of 1 mgm histamine*

		Normal	After histamine injections
Blood cells, total	volumes per cent	47.8	45.2
RBC, only	volumes per cent	46.3	43.6
RBC	millions per c mm	6.60	6.58
Erythrocyte size.	cu mu	70.1	66.2
Serum protein	grams per 100 cc	7.0	10.8
Nonprotein nitrogen	mgm per 100 cc	25	64
Sugar	mgm per 100 cc	90	50
CO <sub>2</sub> content, plasma	mM per liter	22	38.5
Chloride, plasma	mM per liter	108.5	89
Phosphorus distribution			
Whole blood			
1 Inorganic	mgm per 100 cc	2.38	4.76
2 Total acid-soluble	mgm per 100 cc	27.2	37.7
3 Organic "Ester-P"	mgm per 100 cc	24.82	32.94
4 TOTAL	mgm per 100 cc	42.5	59.3
5 Acid-insoluble	mgm per 100 cc	15.3	21.6
Ester-P RBC count ratio		3.75	5.00
Plasma			
1 Inorganic	mgm per 100 cc	2.72	6.9
2 Total acid soluble	mgm per 100 cc	3.33	7.25
3 Organic "Ester-P"	mgm per 100 cc	0.6	0.35
4 TOTAL	mgm per 100 cc	12.4	25.5
5 Acid-insoluble	mgm per 100 cc	9.07	18.25
Cells			
1 Inorganic	mgm per 100 cc	2.01	2.17
2 Total acid soluble	mgm per 100 cc	53.2	74.6
3 Organic "Ester-P"	mgm per 100 cc	51.2	72.4
4 TOTAL	mgm per 100 cc	75.4	100.3
5 Acid-insoluble	mgm per 100 cc	22.1	25.7

taken before operation were not made, in the first column of Table 2 are listed for comparison the figures for nonprotein nitrogen,  $\text{CO}_2$  content, Cl and phosphorus distribution determined in the blood of a normal dog which had approximately the same relative cell volume, cell count, and hemoglobin. In the blood sample taken just before death (second column, Table 2) note especially the following values (1) elevated serum protein and nonprotein nitrogen, (2) the low Cl and high  $\text{CO}_2$  content of the plasma, (3) the increased phosphorus content of the cells, the increase being practically all in the ester-P fraction. In this last blood sample were found values of such magnitude of change from the normal as have been observed in the blood of dogs with simple pyloric obstruction (i.e., without histamine injections, or other treatment) only at 48 to 72 hours or longer after operation, in this dog these changes developed in the brief course of 19 hours after operation, or only 16 hours after the histamine injections were started.

*Normal dog injected with histamine, followed by pyloric obstruction and repeated histamine injections. Dog number 272 (Table 3)*

A normal dog was subjected to hourly subcutaneous injections of 1 mgm of histamine, in 1.0 cc of 0.9 per cent NaCl solution, for 48 hours. Food was withheld. Throughout this period the animal manifested no outward signs of ill effects of the injections at any time. Blood samples were taken for analysis before the injections were started and again at 24 and 48 hours. After 16 days of rest a pyloric obstruction was created and immediately after recovery from the ether anesthesia the dog was subjected to the same hourly subcutaneous injections of histamine as before. Blood samples were again taken at the intervals indicated in the table. Since it was undesirable to draw large blood samples, complete analyses were not done on the preliminary blood samples taken before each of these experiments. The injections of histamine in the unoperated animal were almost without significant effect on the blood. There was a slight but measurable diminution in size of the erythrocytes, 65.5 to 62.4 cu. microns, and a slight diminution of the chloride, more noticeable in the cells. The ester P of the cells increased slightly. After pyloric obstruction, 16 days later, the same injections of histamine were attended by rapid changes in the blood, as follows: (1) Concentration of the blood, indicated by the increasing red blood cell count and increased serum protein. (2) Loss of chloride and increase of  $\text{CO}_2$  in both plasma and cells. (3) Increases of the total phosphorus, practically all in the ester P fraction in the cells. These changes were in no wise different from those observed in other dogs with simple pyloric obstruction, but developed much more rapidly. In the last blood sample, taken 1 hour before death (22 hours after operation) after 16 injections of 1 mgm doses of histamine, the magnitude of changes in the blood is, as in the previous experiment,



TABLE 3

## Dog number 272

Normal dog received hourly subcutaneous injections of 1 mgm histamine in 0.9 per cent NaCl. Appeared to suffer no ill effects. Pyloric obstruction. Operation performed 16 days later. Five hours after the operation, started hourly subcutaneous injections of 1 mgm doses of histamine as before. The dog died 26 hours after the operation.

	Normal period					Pyloric obstruction		
	Before histamine	Hourly doses 1 mgm histamine		Before operation		Histamine 1 mgm hourly started 6 hours after operation		
		Hours after start of injections				Hours after operation		
		24	48			12	19	22
Weight		22.2		16	23.6			20.1
Blood cells, total	<i>kilos</i>	42.3		Days	45.2	48.2	53.0	53.1
RBC, only	<i>volumes per cent</i>	41.3		Rest	43.2	46.0	50.6	50.7
RBC	<i>millions per c mm</i>	6.30	6.50		6.62	7.97	9.72	9.82
Erythrocyte size	<i>cu mm</i>	65.5	64.6		65.2	57.7	52.0	51.6
Hemoglobin whole blood	<i>grams per 100 cc</i>	13.56	13.81			16.63	19.51	19.11
Hemoglobin cells	<i>grams per 100 cc</i>	32.8	32.9			36.1	38.5	37.7
Hb RBC count ratio		2.15	2.12	2.07		2.08	2.00	1.95
Serum protein	<i>grams per 100 cc</i>	7.9	6.8	5.8				10.1
Nonprotein nitrogen	<i>mgm per 100 cc</i>		15	17				
Sugar	<i>mgm per 100 cc</i>		70	67				
CO <sub>2</sub> content, plasma	<i>mM per liter</i>	22.0*	25.1	25.0	22.0*	36.1	17.2	11.1
CO <sub>2</sub> content, cells	<i>mM per liter</i>	13.5*	15.3	20.9	13.5*			45.2
Chloride, plasma	<i>mM per liter</i>	106.0	103.0	105.0	106.0	91.0	77.5	21.6
Chloride, cells	<i>mM per liter</i>	58.0*	56.9	53.4	58.0*	10.2	36.0	73.5
								32.0

TABLE 3 (continued)

		Normal period						Pyloic obstruction		
		Before histamine	Hourly doses 1 mgm. histamine		Before operation	Hours after operation	Histamine 1 mgm. hourly started 6 hours after operation			
			Hours after start of injections				Hours after operation			
			24	48			12	19	22	
			16 Days Rest							
Phosphorus distribution										
Whole blood										
1 Inorganic	mgm per 100 cc		4.00	5.52						4.47
2 Total acid soluble	mgm per 100 cc		27.4	27.2						48.3
3 Organic 'Ester P'	mgm per 100 cc		23.4	21.68						43.83
4 TOTAL	mgm per 100 cc	43.5	41.8	44.5	50.6		65.6			73.4
5 Acid insoluble	mgm per 100 cc		14.4	17.3						25.1
Ester P RBC count ratio		3.5*	3.60	3.67	3.5*					4.46
Plasma										
1 Inorganic	mgm per 100 cc		6.02	6.78						6.73
2 Total acid soluble	mgm per 100 cc		6.43	6.8						7.22
3 Organic 'Ester P'	mgm per 100 cc		0.4	0.0						0.5
4 TOTAL	mgm per 100 cc	20.2	20.2	19.4	22.6		27.4			31.4
5 Acid insoluble	mgm per 100 cc		13.8	12.6						23.8
Cells										
1 Inorganic	mgm per 100 cc		1.36	3.45						2.47
2 Total acid soluble	mgm per 100 cc		54.7	60.7						84.5
3 Organic "Ester P"	mgm per 100 cc	52.0*	53.4	57.3						82.1
4 TOTAL	mgm per 100 cc	75.3	69.9	85.8	84.5		99.5			110.8
5 Acid insoluble	mgm per 100 cc		15.2	25.0						26.2

\* Assumed normal values, taken for comparison

approximately that seen in dogs with simple pyloric obstruction at 48 to 72 hours, or longer, after operation

*Pyloric obstruction + salt solution + histamine Dog number 297*  
(Tables 4 and 5)

In dogs with simple pyloric obstruction the parenteral administration of NaCl solution in appropriate amounts (around 50 cc or more per kilo) prolongs life in the animals and diminishes the alterations of the blood,

TABLE 4

*Time schedule of the experiment shown in Table 5 for dog number 297*

Hours after operation	0.9 per cent NaCl solution	Number of blood sample	Notes
	"	1	Weight before operation, 21 kgm Sample taken 24 hours before operation
1	1500	2	Histamine 1 mgm in 1 cc 0.9 per cent NaCl solution, hourly injections started immediately after 2nd blood sample was taken, and continued through 48 hours
22	1000		
30		2	
36	1350		
48	1000	3	
52			
54	1500	4	
60	1200		
72	1000		
78			

as described in the first paragraph of this paper. If the effect of histamine in hastening death in obstructed dogs is mainly one of stimulating gastric secretion and thus accelerating the losses of chloride from the body, then the administration of salt solution in sufficient amounts theoretically should protect these dogs as well as those with simple obstruction. The following experiment was performed to determine whether such protection could be obtained.

In a dog weighing 21 kgm the pylorus was obstructed, under ether anesthesia, with the usual technic. Immediately after operation 1500 cc of salt solution were given subcutaneously. Again at 22 hours after operation 1000 cc of salt solution were given. At 30 hours after operation a blood sample was taken and immediately afterwards hourly subcutaneous injections of 1 mgm histamine in 1.0 cc 0.9 per cent NaCl solution were started. (This interval of 30 hours before starting the histamine injections was allowed so that the dog might recover from the immediate effects of the anesthesia and operation. A repetition of this experiment, in which both the histamine injections and the parenteral administration of salt solution were started immediately after operation, gave almost exactly the same results as shown here, so this time interval appeared to

TABLE 5

*Dog number 297 Pyloric obstruction + salt solution hourly injections of histamine for 48 hours*

Hours after operation Number of blood sample		Before 1	30 2	52 3	78 4
Blood cells total	volumes per cent	44.2	49.9	48.4	40.9
RBC, only	volumes per cent	43.1	48.3	46.8	39.6
RBC	millions per c mm	6.45	8.20	8.50	6.45
Erythrocyte size.	cu. mu	66.8	58.9	55.0	61.4
Hemoglobin whole blood	grams per 100 cc	15.76			15.44
Hemoglobin cells	grams per 100 cc	36.5			39.0
Hb RBC count ratio		2.44			2.39
Serum protein	grams per 100 cc	7.8	10.4	8.72	7.44
Nonprotein nitrogen	mgm per 100 cc	30*		20	25
Sugar	mgm per 100 cc	90*		85	59
CO <sub>2</sub> content plasma	mM per liter	22.0*		32.1	32.8
CO <sub>2</sub> content, cells	mM per liter	13.0*		17.4	18.4
Chloride plasma	mM per liter	111.0	111.5	113.0	114.5
Chloride cells	mM per liter	60.1	53.4	40.7	59.5
Phosphorus distribution					
Whole blood					
1 Inorganic	mgm per 100 cc	3.12	3.02	2.7	3.24
2 Total acid-soluble	mgm per 100 cc	24.6	32.3	34.5	29.4
3 Organic "Ester P	mgm per 100 cc	20.5	29.3	31.8	26.16
4 TOTAL	mgm per 100 cc	40.0	48.7	51.5	45.0
5 Acid insoluble	mgm per 100 cc	15.4	16.4	17.0	15.6
Ester P RBC count ratio		3.18	3.57	3.74	4.05
Plasma					
1 Inorganic	mgm per 100 cc				2.92
2 Total acid-soluble	mgm per 100 cc				4.32
3 Organic "Ester P	mgm per 100 cc				0.4
4 TOTAL	mgm per 100 cc	14.6	17.1	17.4	15.0
5 Acid insoluble	mgm per 100 cc				10.7
Cells					
1 Inorganic	mgm per 100 cc				2.26
2 Total acid-soluble	mgm per 100 cc				65.6
3 Organic Ester P	mgm per 100 cc	47.5†	58.7†	65.7†	63.4
4 TOTAL	mgm per 100 cc	72.0	80.4	87.8	88.3
5 Acid insoluble	mgm per 100 cc				22.7

\* Assumed normal values, taken for comparison

† Where the complete analyses of plasma were not made, the organic ester P of the cells was calculated without allowing for the slight amount of ester-P in the plasma. The error thus introduced is negligible

be unimportant.) Salt solution was again given at 36 hours and 48 hours, and another blood sample was taken at 52 hours after operation (See the time schedule of the experiment in Table 4) In this blood sample No. 3 the cell chlorides were found to be low and in the next 24 hours the administration of salt solution was increased (See Table 4) In

the last blood sample, at 78 hours, the chlorides of both plasma and cells were again at practically the initial level. The nonprotein nitrogen had remained normal. The total phosphorus of the cells increased, and the figure for the *Ester-P RBC count ratio* steadily increased, but these increases in the cell P are not nearly as great as those shown after simple pyloric obstruction in Table 1. The changes in the plasma phosphorus were negligible.

The dog was sacrificed after the blood sample taken 78 hours after operation (48 hours of histamine injections). The experiment had been continued long enough to demonstrate that the dog could be kept alive by the parenteral administration of salt solution well beyond the time at which death occurred in dogs with obstruction, similarly injected with histamine but not receiving salt solution. Were it not for the exigencies of the experiment—the large blood samples that had been taken, etc—it seems likely that this dog could have been kept alive for even a longer time by this treatment.

#### DISCUSSION

In these experiments it is demonstrated that when dogs with pyloric fistula or pyloric obstruction are injected with small repeated doses of histamine, they develop more rapidly all the chemical changes of the blood which are ordinarily associated with intestinal obstruction, and die much more quickly than do the untreated dogs with simple obstruction. In normal unoperated dogs similar injections of histamine continued for even longer periods were without visible deleterious effects and caused only slight changes in the blood. Histamine thus injected is known to have a powerful effect in stimulating the secretion of gastric juice. In normal animals these secretions presumably pass through the pylorus into the intestine to be reabsorbed and again form part of the body fluids. However, in animals with obstruction and vomiting, such stimulation of the gastric secretion must accelerate the losses of gastric secretions from the stomach and therefore hasten the development of all the chemical changes which occur in the body in consequence of these losses. If one accepts the existing evidence that the alterations of the blood and body fluids ordinarily observed in simple high obstruction are due mainly to the losses of water and electrolytes by vomiting, it seems reasonable to believe that the same mechanism is operative in producing the altogether similar changes that are observed when dogs with obstruction are injected with histamine. It seems unnecessary to postulate a general toxic action of the histamine on the body tissues to explain the effects of histamine in hastening death in the obstructed animals. The immediate death with the symptoms of "shock" that follows the intravenous injections of larger doses of histamine may be due to a different mechanism.

Work is in progress to determine whether or not the effects of subcutaneous injections of the toxic substances (proteoses or amines?)

from the contents of the obstructed or strangulated intestine, or from closed loops in dogs, are closely analogous to this effect of histamine. The absorption of such substances conceivably might be sufficiently rapid to cause immediate death with manifestations like those observed after the intravenous injection of these toxic substances, but in clinical experience such a circumstance is certainly exceptional. It is likely that the slow absorption of these substances will be found to have an effect similar to that of histamine in stimulating the flow of gastric juice (Dragstedt and Dragstedt (1922)), if this be true, then it is possible that the most important effect of the slow absorption of toxic substances from a strangulated portion of bowel is not any general "intoxication" of the body tissues, but an acceleration of all those secondary effects which ultimately cause death.

In dogs with obstruction, injected with histamine, much more salt solution was necessary to maintain the blood chlorides at a normal level than in similarly obstructed dogs not receiving histamine injections. In Table 4 it is to be noted that even though the plasma chloride remained at a normal level in the obstructed dog receiving large amounts of salt solution parenterally along with the hourly injections of histamine, the cell chloride fell markedly, from 60.1 mM before operation to 53.4 and 40.7 mM, in the blood samples taken at 30 and 52 hours, respectively, after operation. A larger amount of salt solution was given in the 24 hours before the last blood sample was taken, 78 hours after operation, and in this blood the cell chloride was again at the normal level. This observation of a fall in the chloride greater in the cells than in the plasma after histamine injections has been confirmed in several experiments, and was observed by Lim and Ni (1926) in their experiments mentioned in the early part of this paper. In several of the experiments with obstructed dogs, both with and without histamine injections, it has seemed to be more difficult to prevent the losses of Cl from the blood cells and the increases of ester-P in the cells than to maintain the plasma Cl at a normal level by means of the parenteral administration of NaCl solution. Much more work is needed to ascertain the significance of this in relation to treatment.

#### SUMMARY

In dogs with pyloric obstruction, repeated subcutaneous injections of small doses of histamine have the effect of hastening the development of those chemical changes in the blood which have been claimed by many to be the most important ultimate cause of death.

Histamine has been employed as a known substance which is at least closely related to the toxic substances which appear in the contents of a segment of obstructed or strangulated bowel. Histamine, injected subcutaneously as in these experiments, stimulates the flow of gastric juice

and in the presence of vomiting such increased secretion results in more rapid losses of electrolytes and water than occur in animals with simple obstruction, in consequence of this there is a more rapid development of the whole cycle of the symptoms and chemical changes which ordinarily accompany intestinal obstruction

From these experiments it is suggested that patients with intestinal obstruction plus strangulation, or dogs with experimental obstruction plus closed loops, die sooner than do those with simple obstruction not because of any specific general "intoxication" of the body tissues, but because the slow absorption of histamine-like substances from the bowel results in an acceleration of the progress of all those chemical changes which occur secondarily to the losses of gastric secretions

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## THE SPINAL FLUID IN HYPERTENSION<sup>1</sup> \*

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(Received for publication December 12, 1931)

This study was originally prompted by a very disturbing result of lumbar puncture in a patient who had headache, severe hypertensive disease and marked eyeground changes. Shortly after the puncture the patient became definitely psychotic. This brought to a head certain questions previously entertained concerning the intracranial pressure in this disease, how often the headache could be attributed to increased pressure within the skull, and when lumbar puncture could be used as a therapeutic measure.

We knew from the experience of others and our own earlier work that not every case of hypertension was accompanied by elevation of spinal fluid pressure. Our chief endeavor was to establish some method of determining which patients had such increased pressure without actually performing the lumbar puncture. If such an indirect method could be established, it would be possible often to avoid an unnecessary, trouble some and possibly harmful procedure.

Although many authors had written on this subject their conclusions were far from uniform. We will mention only a few papers to show the varying trend of ideas which confronted us in the literature. The earlier writers, notably Lyttkens (1), suggested on very scant evidence that a rise in blood pressure was followed by a rise in the cerebrospinal fluid pressure. Later work, principally by French investigators (2), seemed to demonstrate that there is no direct relationship between the pressures in the two systems. However, Claude and Lamache (2), Block and Oppenheimer (3), maintained that after an equilibrium has been established as in a patient with long standing hypertension, changes in the pressure of one system will be followed by similar changes in the other. From this it seemed fair to assume that an artificially produced reduction in the spinal fluid pressure might be followed by a fall in the blood pressure.

Previous investigators had made no effort to correlate the appearance of the optic disc, the clinical symptoms and the type of hypertension with the cerebrospinal fluid pressure. Larsson (4) studied the spinal fluid pressure of eleven patients who had choked discs associated with nephritis.

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He found in all of his cases that papilledema was accompanied by an increased intracranial pressure. Fishberg and Oppenheimer (5), however, in their recent paper have suggested that the association of papilledema with increased cerebrospinal fluid pressure was not so constant as Larsson pointed out.

TABLE I

*Findings on 22 cases of hypertensive disease that showed either edema of the discs or increased cerebrospinal fluid pressure\**

Name	Diagnosis	Systemic blood pressure	Cerebrospinal fluid pressure	Papilledema†	Renal insufficiency	Cardiac decompensation
		mm Hg	mm of H <sub>2</sub> O			
G O	Hypertension	208/136	240	++	0	0
S W	Hypertension	210/145	280	++	0	0
	Ruptured gastric ulcer					
R W	Hypertension	215/140	230	++	0	0
E M	Hypertension	235/130	310	++	severe	severe
	Chronic nephritis (vascular)					
E H	Hypertension	250/150	300	++	mild	mild
	Chronic nephritis (vascular)					
E A	Hypertension	280/150	290	++	severe	severe
	Chronic nephritis (vascular)					
B S	Hypertension	215/135	480	++	severe	mild
	Chronic nephritis (vascular)					
D O	Hypertension	215/145	305	++	mild	0
N W	Hypertension	210/140	290	+	0	mild
	Asthma					
D F	Hypertension	210/105	290	+	severe	mild
	Chronic nephritis (vascular)					
B B	Hypertension	242/130	260	+	0	0
P L	Hypertension	180/140	220	+	0	0
L L	Hypertension	220/120	260	+	0	mild
A M	Hypertension	230/120	330	+	0	0
N B	Hypertension	240/130	225	+	0	0
T L	Hypertension	230/120	255	+	0	0
C C	Hypertension	215/125	280	+	0	0
A R	Hypertension	220/140	230	0	0	0
	Edema of brain					
A O	Chronic nephritis with edema	210/150	300	++	severe	0
A C	Acute nephritis	140/105	235	++	mild	0
J R	Chronic nephrosis	158/102	130	+	mild	0
T W	Chronic nephritis (glomerular)	165/120	180	++	severe	mild

\* The other 28 cases in this series are omitted for the sake of brevity. Although they were patients with hypertension, they showed neither edema of the disc nor increased cerebrospinal fluid pressure.

† + indicates physiological cup filled, indistinct disc margins, marked blurring of the disc but no elevation of the disc.

++ indicates definite elevation of the disc from 1 to 5 diopters (choking).

## METHOD OF STUDY

Fifty patients with well marked hypertensive disease were selected. We avoided as far as possible cases with advanced cardiac congestive failure. Blood pressure readings had been made for several days before lumbar puncture was done. Readings were also made immediately before, during, and thirty to forty five minutes after the puncture. Occasionally, another determination was made twelve hours later.

The lumbar punctures were made in the lateral recumbent position, exercising considerable care in "levelling" the patient. Water manometers were used and no reading was made until the oscillations accompanying the heart beat and respiration were stabilized. A reading of less than 200 mm. was considered normal. In those patients with high intracranial pressures, care was exercised not to lower this pressure below 200 mm. Cell counts, protein estimations, Wassermann and colloidal gold tests were made on all fluids. The results were normal. Chlorides were determined by the method of Whitehorn (10) in a sufficient number to indicate that these 50 were probably all normal.

Careful examination of the eyegrounds was made in every case. Roentgenograms of the skull were done in 32 cases. Renal function was determined by means of the phthalein test and the determination of the blood urea nitrogen.

The results of this study as a whole are outlined in Table I.

The relation of edema of the optic discs to the cerebrospinal fluid pressure is indicated in Table II.

TABLE II  
*Intraspinal pressure and papilledema*

Spinal fluid pressure		With papilledema
	<i>Number of cases</i>	<i>cases</i>
Normal	30	2
Increased	20	19

Out of 20 cases with increased spinal fluid pressure 19 disclosed papilledema. On the other hand, of the 30 with normal spinal pressures only 2 had papilledema.<sup>1</sup>

There were 4 cases which showed slight blurring of the discs with no filling of the physiological cup and no venous congestion. In all of these

<sup>1</sup> One of these two had the syndrome of nephrosis with early glomerulonephritis and while under our observation developed papilledema. Spinal fluid pressure was only 130 mm. of water. The other had chronic glomerulonephritis with choking of both discs but spinal fluid pressure was 180 mm. of water. This patient had a cellulitis in back of his left eye which may have contributed to the swelling of the nerve head.

the intracranial pressure was normal. These are not classed as true papilledema.

TABLE III

*Papilledema and intraspinal pressure*

Papilledema with no measurable choking	1 case 130 mm 7 cases 230/300 mm 1 case 330 mm
Papilledema with choking of one to five diopters	1 case 180 mm 6 cases 230/300 mm 5 cases over 300 mm

Table III shows some correlation between the degree of papilledema and the intracranial pressure. The patients with mild papilledema as a rule had lower intracranial pressures than those with a greater degree of papilledema.

TABLE IV

*Renal insufficiency*

Renal function		Increase spinal pressure	
	Number of cases	cases	per cent
Normal	34	11	32
Depressed	16	10	63

TABLE IVa

*The relation of the degree of papilledema to the degree of renal impairment*

Papilledema	
10 with less than 1 diopter choking	{ 8 cases with normal function { 1 case with mild insufficiency { 1 case with marked insufficiency
11 with 1 to 5 diopters choking	{ 3 cases with normal function { 3 cases with mild insufficiency { 5 cases with marked insufficiency

In Table IV we see that papilledema occurred in 32 per cent of patients with normal renal function but in 66 per cent of those with impaired renal function. Moreover, in Table IVa we see that the degree of renal impairment is greater in those cases with more severe papilledema. In view of the work of Fishberg and Oppenheimer (5), Weiss and Ellis (6) and others we were not surprised at the high incidence of papilledema in those patients with hypertensive disease who have progressed to renal impairment. Of particular significance, however, is the fact that 11 patients with normal renal function had papilledema and increased intracranial pressure.

In Table V we see that headache occurs more frequently in those cases which have increased intracranial pressure and this at first suggests the pressure as a causative factor in the headache. But since 40 per cent

TABLE V  
*Correlation of spinal fluid pressure with headaches*

Spinal fluid pressure		Without headaches		With headaches	
	Number of cases	cases	per cent	cases	per cent
Normal	30	18	60	12	40
Increased	20	4	20	16	80

of those with normal pressure also had headache, some other factor must certainly be present. It is possibly significant that 8 of the 12 with headache in the group with normal intracranial pressure had very high diastolic blood pressures.

Headache was relieved after lumbar puncture in only two cases and in three cases severe headaches followed the procedure. Cushing and Bordley (7) in 1908 reported temporary relief by subtemporal decompression of a partial blindness in a patient with chronic nephritis and choked discs. Larsson (4) showed a definite improvement in visual acuity in some of his cases on lumbar drainage. We studied two cases on the services of Dr. Cushing and Dr. O'Hare, who had marked diminution of visual acuity as a result of choked discs. Both had headaches and severe hypertensive vascular disease. The vision of one of these patients was relieved for about seven months following subtemporal decompression. The other had relief for a shorter period but died a few weeks after the decompression from ruptured peptic ulcer. It seems that while headache may be in part due to increased intracranial pressure, lumbar drainage for relief of headache is not justified. However, it appears from a study of Dr. Cushing's cases that subtemporal decompression in certain selected cases in the hands of a competent surgeon may be justified in the presence of advanced visual failure.

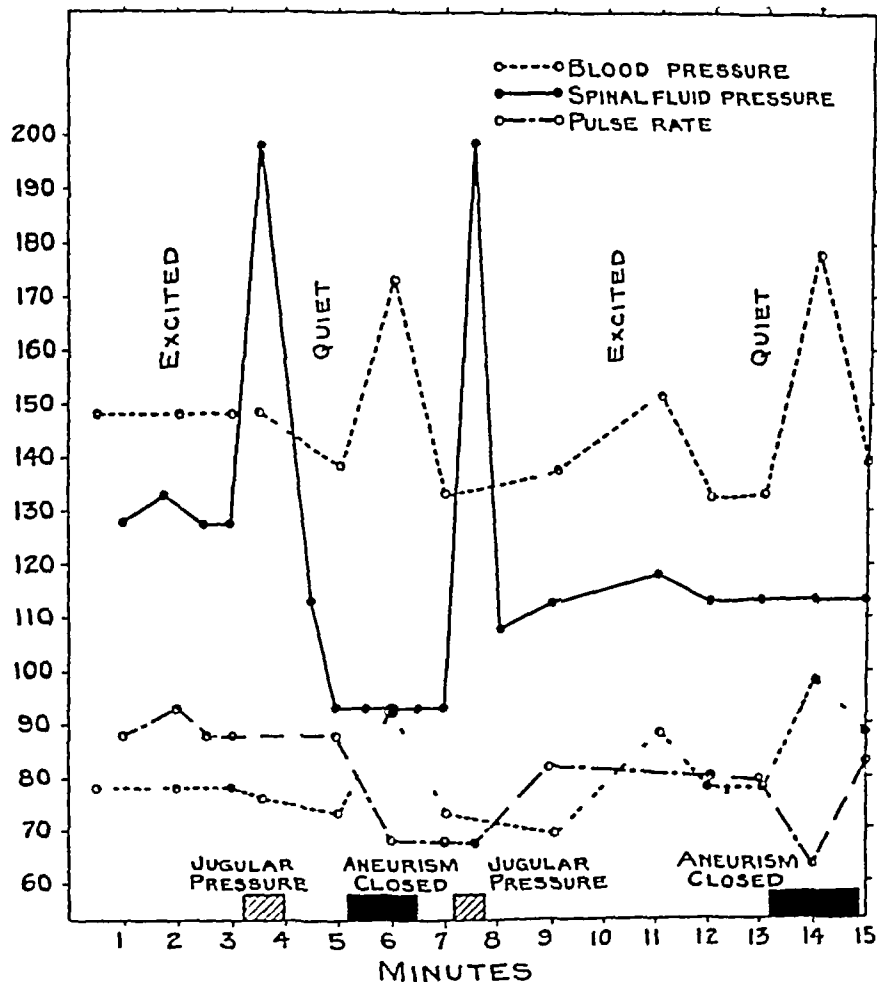
TABLE VI  
*Correlation of spinal fluid pressures with systolic and diastolic arterial pressures*

Spinal fluid pressure		Blood pressures					
		Average		Number systolic above 220		Number diastolic above 130	
		Systolic	Diastolic				
	Number of cases	mm. Hg	mm. Hg	cases	per cent	cases	per cent
Normal	30	198	115	10	33	8	31
Increased	20	219	135	9	45	16	80

The results shown in Table VI indicate that the average blood pressure, particularly the diastolic, is greater where spinal fluid pressure is increased. Sixteen out of 20 cases in this group had diastolic pressures

over 130 mm Hg. It is also important to note that 8 out of 30 in the group with normal spinal fluid pressure also had diastolic pressures over 130 mm Hg. We claim no direct causal relation but the frequency of high diastolic pressure is of interest. Weed and Hughson (8) from their work on animals believe there is no connection between the two.

A recent experiment by one of us (S. A. S.) at Lakeside Hospital in Cleveland is pertinent to this discussion. A patient with a right femoral arteriovenous fistula was studied. Occlusion of the fistula produced a rapid rise in the blood pressure, both systolic and diastolic. Chart I



#### THE EFFECT OF RAPID ELEVATION OF THE SYSTEMIC BLOOD PRESSURE ON THE SPINAL FLUID PRESSURE

The elevation of the blood pressure is accomplished by the closure of a femoral arteriovenous aneurism.

shows the result of the studies of the spinal fluid pressures during the rise in blood pressure. We see here the typical rise in the spinal fluid pressure with jugular compression but no change during the rapid rise in arterial pressure.

The records of the blood pressure changes during and after reduction of spinal fluid pressure show a fall of 15 mm Hg in either systolic or diastolic blood pressure in only 9 cases. Changes of less than 15 mm may be easily accounted for by the relief and relaxation which the patient experiences after the needle is in. (The blood pressure in a case of meningitis in coma was followed very carefully as the lumbar region was drained. The pressure of 140/70 did not vary during the lowering of the spinal fluid pressure from 450 mm of water to 25 mm.)

Roentgenological changes in the skull. X-rays of the skull in 32 cases were made. Eleven of these had increased intracranial pressure as measured by the manometer but only 2 showed changes in the skull visible in x-ray. These signs were identical with those found with high intracranial pressures accompanying brain tumor.

In this short series of cases we can throw no light on the mechanism involved in the causation of increased intracranial pressure. Chemical studies of the fluid were negative throughout, one of two cases which came to autopsy showed edema of the brain, a majority of the increased pressures were accompanied by high arterial diastolic pressures, and there is a group of 7 cases which had neither chronic nephritis as a cause for choked disc, nor cardiac compensation with its associated increased venous pressure (6) (9).

#### SUMMARY AND CONCLUSIONS

1 A study of 50 cases of hypertensive disease showed 21 to have increased intracranial pressure.

2 Papilledema was almost always associated with increased intracranial pressure.

3 Papilledema and increased intracranial pressure occur more frequently with renal failure but are also found where renal function is normal.

4 Headache is more frequent in the presence of increased intracranial pressure and papilledema but occurs without either one. Our results indicate that lumbar drainage for relief of headache is not justified.

5 Increased intracranial pressure seems more often associated with high diastolic blood pressure but we feel that both are probably the result of some common factor and neither is caused by the other.

6 The cause of increased intracranial pressure is not accounted for in 50 per cent of our cases which have neither renal insufficiency nor increased venous pressure.

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# THE INFLUENCE OF EXPERIMENTAL THYROID INTOXICATION ON THE POTASSIUM, SODIUM, AND WATER CONTENT OF THE MYOCARDIUM

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Harrison et al (1) analyzed samples of cardiac and skeletal muscle obtained from patients who had died of congestive heart failure and found them to be abnormally poor in potassium. It is their belief (2, 3) that this diminution in the potassium content is related to overwork and the attendant fatigue. The heart in experimental thyrotoxicosis seemed to offer a possible opportunity of examining the hypothesis in question under controlled conditions. In addition, the effect of thyroid intoxication on the potassium content of the greatly enlarged heart has an interest of its own.

Thirty six male albino rats 140 days old and 14 rats 100 days old were divided into two groups on the basis of body weight. For two weeks they all received a special diet described elsewhere (4). In addition the diet of one group had 0.2 per cent of desiccated thyroid (Wilson Laboratories) intimately mixed with it. At the end of the period the rats were etherized and the heart removed, freed of adherent blood by blotting on filter paper, weighed on an analytical balance, and preserved for analysis. The water content of the tissue was determined by drying to constant weight at 80° C in vacuo. The residue was then ashed with the aid of nitric acid and heat. Either sodium or potassium was determined on the ash, the small quantity of sample precluding both determinations upon a single heart. For sodium the modification of the uranyl zinc acetate method described by Butler and Tuthill (5) was used and for potassium the volumetric modification of the chloroplatinate method described by Shohl and Bennett (6). The potassium content was determined in the hearts of 15 animals of each group and the sodium content measured in those of the remainder.

The results have been tabulated in Table I. The administration of thyroid substance, although producing the usual marked increase in the weight of the heart, was without demonstrable effect upon the water, potassium or sodium content of the myocardium.

The data tabulated here give no support to the idea that overwork or fatigue causes a diminution in the potassium content of cardiac muscle insofar as the effect of thyroid substance is concerned. They



TABLE I  
The influence in the albino rat of experimental thyroid intoxication upon the potassium, sodium and water content of the myocardium

Number	Controls						Thyroid fed					
	Body weight		Heart weight	Heart H <sub>2</sub> O	K Per cent wet heart	Na Per cent of wet heart	Body weight		Heart weight	Heart H <sub>2</sub> O	K Per cent wet heart	Na Per cent of wet heart
	Initial	After death	grams	per cent	per cent of fresh tissue	per cent of fresh tissue	Initial	After death	grams	per cent	per cent of fresh tissue	per cent of fresh tissue
1	186	213	598	75.8	0.378		229	228	1053	77.1	0.349	
2	238	258	677	75.8	0.370		*158	176	806	75.2	0.375	
3	220	248	691	75.2	0.358		238	220	1021	75.8	0.317	
4	216	239	691	76.0	0.326		*163	182	879	75.3	0.311	
5	213	223	681	75.9	0.320		*156	164	755	75.4	0.327	
6	261	297	769	75.4	0.322		270	270	1116	77.0	0.296	
7	252	295	754	76.1	0.304		*182	194	945	75.1	0.322	
8	210	228	600	76.0	0.300		185	181	842	75.3	0.317	
9	*191	239	660	75.6	0.302		170	168	811	76.6	0.297	
10	*165	200	626	74.7	0.312		215	226	1044	76.5	0.297	
11	*153	180	526	71.5	0.304		191	197	875	75.2	0.310	
12	*118	176	510	71.6	0.300		214	207	856	75.5	0.301	
13	171	200	621	75.3	0.292		213	194	940	75.5	0.275	
14	*166	208	592	75.0	0.289		213	224	903	75.7	0.256	
15	231	273	772	75.1	0.258		220	210	1118	75.3	0.259	
16	*112	181	527	75.2		0.096	197	213	933	77.9		0.087
17	*110	178	520	71.8		0.088	200	193	915	77.4		0.085
18	220	251	621	75.1		0.086	*128	148	756	76.2		0.086
19	*132	160	510	75.1		0.076	216	210	955	77.0		0.092
20	227	264	698	76.6		0.074	*145	162	846	74.5		0.087
21	225	289	762	75.9		0.068	228	232	1079	76.6		0.078
22	236	294	803	75.7		0.068	200	199	866	77.0		0.076
23	208	222	582	75.2		0.064	235	236	1021	75.6		0.080
24	220	227	715	76.3		0.064	222	221	860	76.3		0.077
25	200	215	656	75.6		0.054	*142	154	680	75.3		0.071
Average	199	234	649	75.6	0.316	0.074	199	200	917	76.1	0.311	0.081

cannot, however, be interpreted as directly opposing this hypothesis proposed by Harrison (2,3 ), for there is some doubt (7) that heart failure ever occurs in goiter with hyperthyroidism as its sole cause. If this is true our assumption that thyroid intoxication produces overwork and fatigue of the myocardium may be incorrect.

The constancy in the potassium content of the heart muscle before and following thyroxinization is very interesting. It would appear to indicate that the increase in heart weight is a simple hypertrophy, the tissue having essentially the same composition after the remarkable weight increase as before the administration of thyroid material.

#### SUMMARY

The increase in heart weight which ensues when active thyroid material is administered to the albino rat is without demonstrable effect upon the potassium, sodium or water content of the myocardium.

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# THE EXCRETION OF XYLOSE AS AN INDEX OF DAMAGED RENAL FUNCTION

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The constancy of the rate of excretion of xylose by normal subjects suggested the possibility of its use as a delicate indicator of damage to renal function. Without entering into the controversy as to the fate of the sugar not accounted for in the urine, it may be stated that the uniformity of the amount excreted in unit time by normal persons following ingestion of a definite quantity of xylose under conditions of fixed fluid intake makes the relative values obtained in patients manifesting impaired kidney function of particular theoretical and clinical significance. The present availability of xylose due to its recently established low cost renders its use for this purpose feasible.

Five grams of purified recrystallized xylose were injected intravenously into a normal subject weighing 75 kilos. The urine was collected by catheter in one minute samples for the first thirteen minutes, and was then voided at intervals of two, four and sixteen hours. It can be seen from Table I that the excretion of xylose starts one minute

TABLE I

*Urine by catheter after injection of 5 grams xylose in a normal subject*

Time	Volume	Per cent	Xylose	Average output per minute
<i>minutes</i>	<i>cc.</i>		<i>grams</i>	<i>grams</i>
1	0.6	0.0	0.0	
2	1.8	0.05	0.0008	
3	3.8	0.1	0.004	
4	1.4	0.4	0.006	
5	1.5	0.4	0.006	
6	3.4	0.3	0.010	
7	1.2	0.5	0.006	
8	1.6	0.5	0.006	
9	2.0	0.4	0.008	
10	1.0	0.8	0.008	
11	1.2	0.7	0.008	
12	1.6	0.5	0.008	
13	3.4	0.27	0.008	
14-120	65	0.8	0.5	0.0047
120-240	240	0.14	0.3	0.0025
240-960	1500	0.3	45	0.0006

Reducing substance determined by method of Hagedorn and Jensen (5)

after injection, to rise within the next three minutes to the rate of 10 mgm per minute during the sixth minute and then fall to 8 mgm per minute for the next 7 minutes. Up to the second hour the rate averages 4.7 mgm per minute, 2.5 during the next two hours and 0.6 mgm for the next twelve hours. Approximately 25 per cent of the amount injected can be accounted for in the first sixteen hours. After five minutes we may assume that the entire amount is circulating in the blood stream because the blood volume calculated on this basis, 5.75 liters for a man weighing 75 kilos agrees well with that determined by the congo red method, 5.48 liters. This method of determining blood volume may prove of some clinical use.

It was found by one of the authors (1) that xylose injected into the marginal vein of a rabbit disappears at a rate proportional to the concentration of the nonfermentable reducing substance present in the blood at any moment or that

$$\frac{dC}{dt} = -aC$$

where  $C$  is the concentration of nonfermentable reducing substance in the blood as determined by the method of Somogyi (2). On integration we get  $C = Ae^{-at}$  where  $a$  is a constant dependent on the unit of time employed and  $A$  must be equal to the initial value when  $t$  is 0. This is the equation governing the velocity of a monomolecular chemical reaction. It is thus possible to determine the concentration of the nonfermentable reducing substance in the blood at any time when its concentration at any particular moment is known, since the logarithm

TABLE II

*Disappearance of nonfermentable reducing substance from blood after injection of xylose in a normal subject*

Time	Nonfermentable reducing substance in blood	$a = \frac{A}{C} \times 10^{-4}$
	mgm per cent	
0	28	
2	54	
5	115	118
60	89	117
120	71	123
180	56	126
240	40.4	119

of the concentration of the foreign sugar is proportional to the time after injection. Using any two values of  $C$  we can eliminate  $a$  and get a numerical value for  $A$ . Thus from Table II

$$\frac{C_2}{C_1} = \frac{115}{89} = \frac{e^{-at_1}}{e^{-at_2}} = e^{a(t_2-t_1)}$$

$$a(t_2-t_1) = \log_e 1.29$$

$$a = 0.046$$

$$A = 123$$

The constancy of  $A$ , as seen in Table II, is then proof both of the constancy of  $a$  and the correctness of form of the equation used. On plotting the values found as percentages of the initial value on semilogarithmic paper it is found that a straight line is obtained (Figure 1)

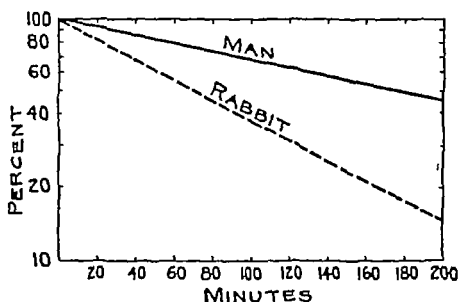


FIG 1 DISAPPEARANCE OF NONFERMENTABLE REDUCING SUBSTANCE FROM BLOOD

After the ingestion of 50 grams of xylose on limited fluid intake and a fasting stomach we found that the blood nonfermentable reducing substance rose to a maximum of approximately 80 within three hours to return to a value of 40 or below within five hours. The return to the original fasting figure is of necessity slow because of the logarithmic nature of the function governing the disappearance of the xylose from the blood.  $25 \pm 5$  per cent of the xylose is excreted within twenty four hours with the majority of normal findings tending to group around 12 to 13 grams. The normal kidney has the power of concentrating xylose to 2.5 per cent within two hours, a point of paramount importance in distinguishing damage to renal function. It can be seen from Figure 3 that the normal kidney excretes 25 per cent of the amount given irrespective of the amount of fluid ingested, since the same quantity was excreted within 24 hours in 554 cc. of urine as in 3450 cc. The balance was so finely adjusted that the excretion in grams per hour was fairly constant whatever the amount of fluid given.

Ten mgm of uranium acetate were injected into a rabbit and the following morning the animal was given 10 grams of xylose by mouth. As can be seen from Figure 4, within five hours the added nonfermentable reducing substance had disappeared from the control rabbit, while the animal treated with uranium retained a high concentration of xylose in the blood for many hours afterward. An animal treated with phosphorus in which the ingestion of 10 grams of galactose produced a prolonged galactose hyperglycemia, was given 10 grams of xylose by mouth. The curve of nonfermentable reducing substance in the blood showed no

deviation from the control. At autopsy the animal showed definite hepatic injury, so it would seem as if damage to liver function does not impair the elimination of xylose.

From a study of Figure 2 it is evident that in patients manifesting kidney lesions the blood curve of nonfermentable reducing substance, instead of approaching the normal fasting value after five hours, continues upward, so that values of 100 mgm per cent or more are encountered. In cases of uremia accompanied by vomiting where some of the xylose

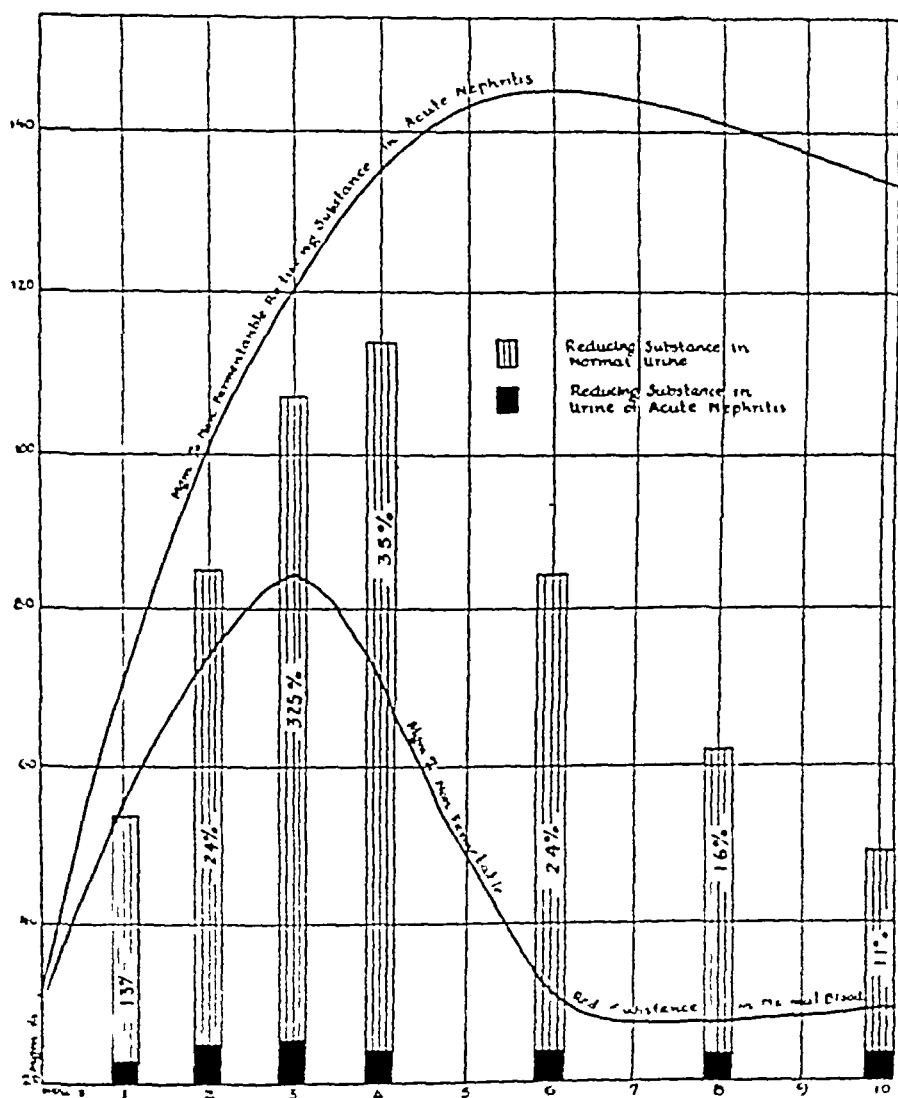


FIG 2 INGESTION OF 50 GRAMS OF XYLOSE BY NORMAL SUBJECT AND BY NEPHRITIC PATIENT

The curves indicate the concentration of nonfermentable reducing substance in the blood, the bars indicate the percentage concentration of reducing substance in the urine.

is lost by the vomiting, it can be seen from Figure 5 that the curve maintains the characteristic shape of poor renal function, though the absolute values reached are never as high. This graph is made from data taken from a case where we were able to recover 22 grams of xylose from the vomitus. For purposes of comparison, a normal subject was given 25 grams of xylose by mouth. It can be seen that the patient with kidney

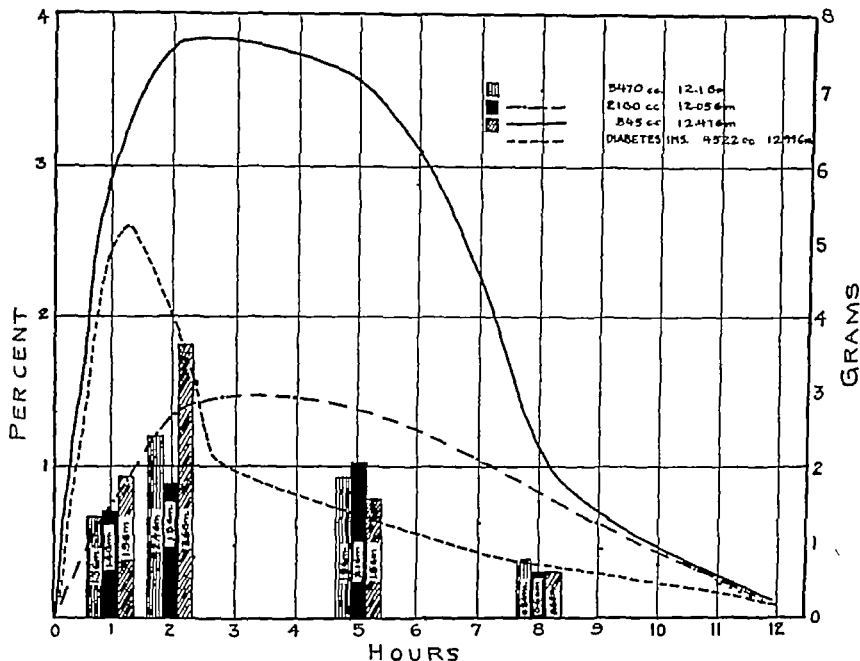


FIG. 3. INGESTION OF 50 GRAMS XYLOSE IN NORMAL SUBJECT ON DIET WITH VARYING AMOUNT OF FLUID

The curves indicate the percentage concentration of reducing substance in the urine; the bars show the output of reducing substance in the urine in grams per hour. For purposes of comparison, the broken line curve indicates the percentage concentration of reducing substance in the urine of a case of diabetes insipidus.

damage shows an entirely different curve. It will be observed in Figure 6 that in a case of acute nephritis which was studied over a period of four months the nonfermentable reducing substance curve of the blood gradually turns down as recovery proceeds, to reach the normal form when kidney function returns to normal. This characteristic shape of the



curve is of special value in incontinent patients where the phenolsulfon-phthalein elimination or the urea clearance cannot be obtained

It has become apparent of recent years that the fundamental manifestation of impairment of renal function is the diminution in the ability of the kidney to eliminate the urinary constituents in high concentration. This is very delicately exemplified in the case of xyllose where the normal

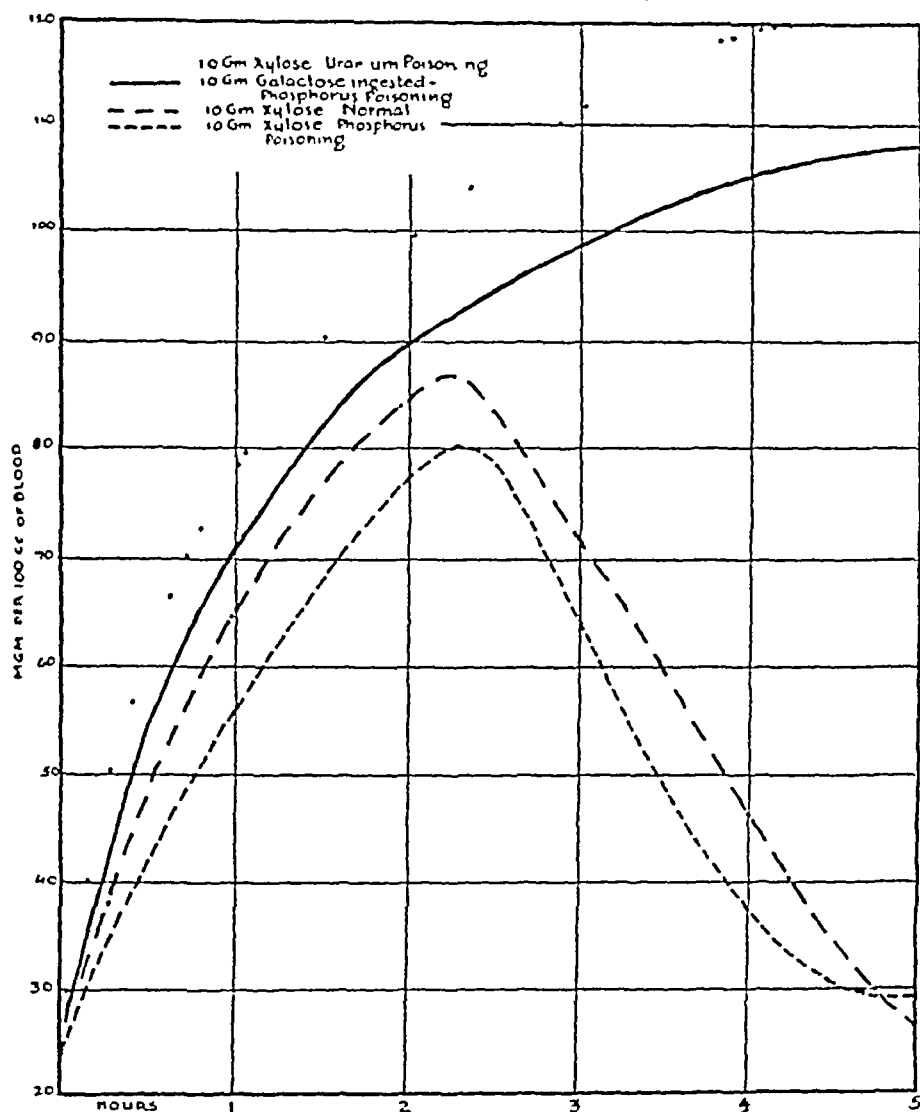


FIG 4 INGESTION OF 10 GRAMS OF NONFERMENTABLE SUGAR IN RABBIT

(— · — ·) indicates the nonfermentable reducing substance in the blood of a normal rabbit after ingestion of xyllose, which does not essentially deviate from that of a rabbit in which experimental acute liver damage has been caused by phosphorus (— — — —). The solid line indicates the curve of the latter animal on ingestion of galactose ( ) indicates the concentration of the nonfermentable reducing substance in the blood of an animal manifesting experimental acute kidney damage caused by uranium

person concentrates to 2.5 per cent or more within two hours after ingestion of 50 grams on limited fluid intake. It is evident from Figure 2 that the subject with damaged kidney function cannot concentrate to this extent. In cases of severe impairment of renal function the concentrating power of the kidney is almost entirely gone, a quantitative Benedict reaction carried out on the urine two hours after ingestion of the xylose shows a concentration of nonfermentable reducing substance of 0.1–0.2 per cent instead of at least twelve times as much in individuals with healthy kidneys. It can be calculated from Figure 2 that the normal

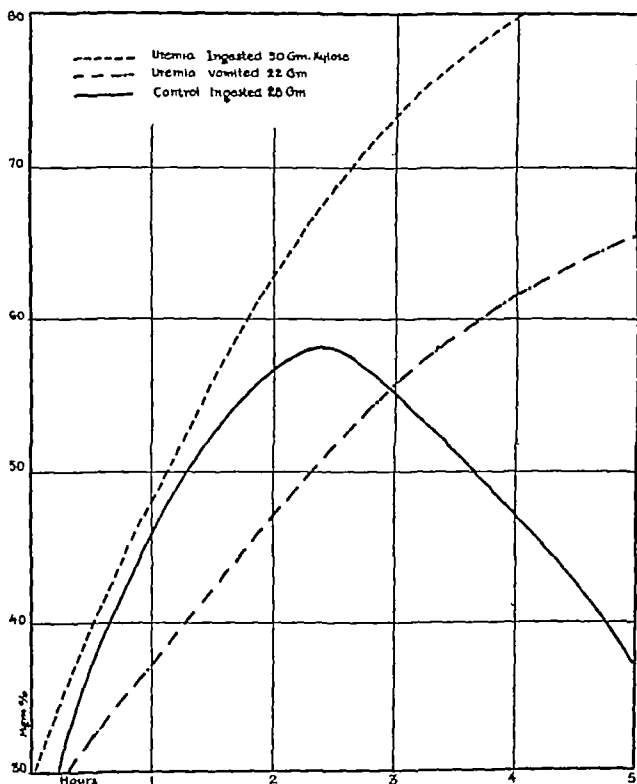


FIG 5 INGESTION OF 50 GRAMS OF XYLOSE BY PATIENT SHOWING MARKED KIDNEY DAMAGE (-----)

Same patient after vomiting 22 grams (— · — · —) Control normal subject, after ingestion of 28 grams xylose (————)

kidney has the power of concentrating xylose from thirty to sixty times while the urine elaborated by the damaged kidney shows a concentration of xylose which is practically equal to that in the blood. This loss in concentrating ability is seemingly correlated with a diminution of the

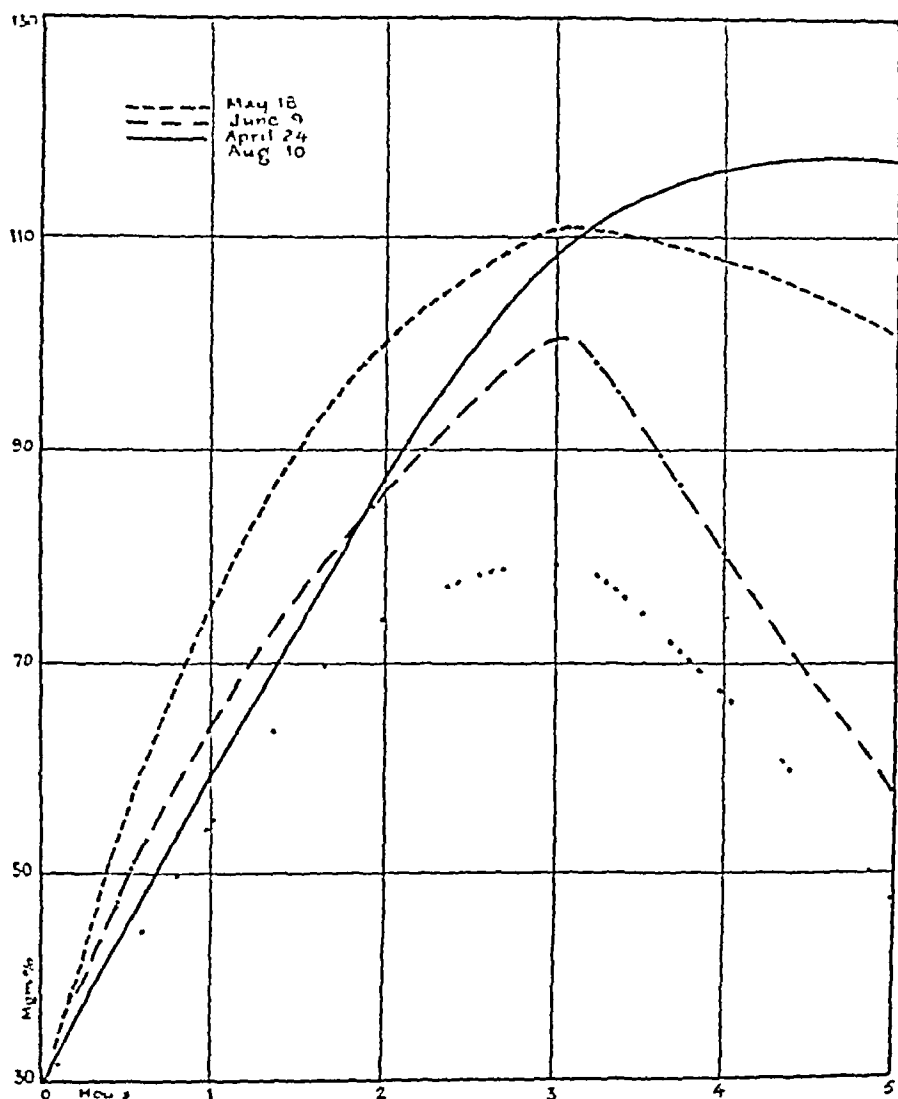


FIG 6 INGESTION OF 50 GRAMS OF XyLOSE AT SUCCESSIVE INTERVALS BY A PATIENT RECOVERING FROM ACUTE NEPHRITIS

The return of kidney function is shown by the change in the form of the curve of the nonfermentable reducing substance in the blood on these successive dates

number of functioning renal units (a renal unit is a glomerulus with its appertaining tubule), but further investigation is necessary to determine in how far this relation can be said to be quantitative, i e., that the intermediate values between the two extremes mentioned above are proportional to the extent of kidney tissue damage

The damaged kidney is not alone unable to concentrate the xylose, but if the amount excreted in twenty-four hours is determined, it is found that this is radically decreased, so that in severe cases of renal disease excretion of one gram or even less is encountered. It is seen that the amount excreted by the normal kidney is a constant independent of the amount of water ingested, so that extrarenal factors may not be of such paramount importance in governing the amount excreted as is the case in other kidney function tests, and the amount of xylose excreted may more closely measure the exact functional permeability of the kidney membrane.

Figure 7 shows a case of acute nephritis in a young girl, which was studied over a period of four months. The comparative renal function tests are represented. It is seen that the ability to concentrate xylose is early and severely impaired, as is the urea clearance. The amount of xylose excreted during 24 hours is also low. The excretion of phenolsulfonphthalein is not so low. This may be an early sign of recovery, since phthalein excretion of the improving patient shows a definite rise sooner, and thereafter rises very quickly toward normal. In the recovery phase it is found in accordance with the work of Peters and Van Slyke (3) that the urea clearance lags behind, so that it is only 50 per cent at a time when the phenolsulfonphthalein excretion is 75 per cent, the xylose concentration is nearly 3 per cent and the patient is able to excrete 11 grams of the fifty grams of xylose ingested.

In Table III the disposition of the xylose in the blood and urine of patients manifesting various types of kidney lesions can be seen.

In the case of J. A. the ability to concentrate to 2.6 per cent within 2 hours and the excretion of 10.81 grams of xylose within 24 hours, as well as the normal xylose curve in the blood, showed intact kidney function. At autopsy, the only kidney lesions found were those of chronic passive congestion. However, in this case, owing to sluggish circulation, the phenolsulfonphthalein excretion was definitely lowered, as was the urea clearance. The specific gravity test was on the border line. In the case of R. A., with valvular cardiac disease, kidney function was intact, as the xylose excretion was 12.24 grams per day and the concentration 3.3 per cent within 2 hours. All the other kidney function tests were in accord with this view.

In a case of diabetes insipidus we found that the patient excreted xylose very quickly, so that if the curve of the concentration of xylose in the urine is compared with those of a normal patient excreting approximately the same volume of urine (see Figure 3) it is seen that the curves are entirely dissimilar. Whether this is characteristic of diabetes insipidus cannot be determined from the one case at our disposal.

In cases of diabetes mellitus it is of course necessary to employ yeast fermentation of the urine to destroy the fermentable reducing substance

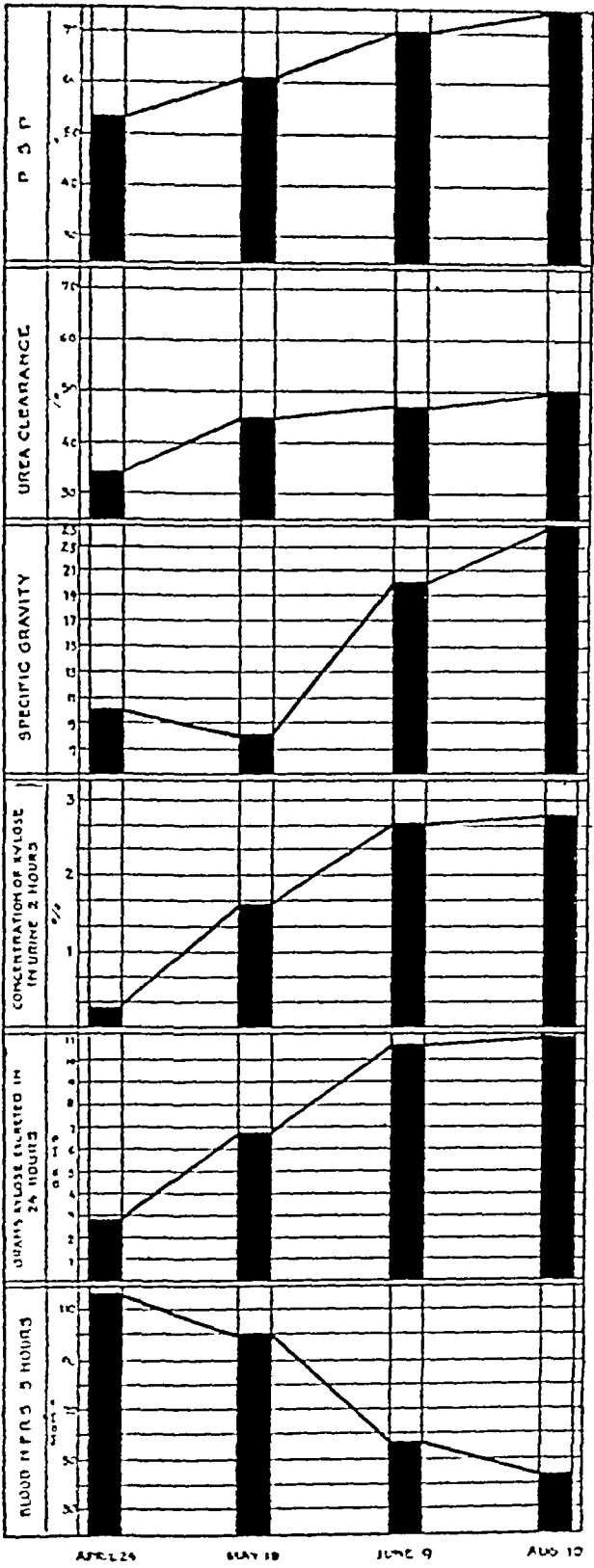


FIG 7 THE UREA CLEARANCE, SPECIFIC GRAVITY OF THE URINE, PHENOLSULFONPHTHALEIN EXCRETION

Also after ingestion of 50 grams of xulose the output in grams in 24 hours, the concentration of the nonfermentable reducing substance in the blood after 5 hours, the percentage concentration of xulose in the urine after 2 hours are indicated at varying periods in a patient recovering from acute nephritis

TABLE III  
*Xylose in blood and urine in patients with kidney lesions*

Name and age	Diagnosis	Blood					Urine													
		Systolic pressure	Red blood cells	Non-protein nitrogen	Xylose				Volume in 24 hours	Urea clearance above	Phosphatase	Specific gravity	Protein	Xylose					Output in 24 hours	
					0 hour	1 hour	3 hours	5 hours						1 hour	2 hours	3 hours	8 hours			
M. B. 50	Arteriod contracted kidney	88	2.2	120	31	69	100	110	777	3"	3	1.010	f.t.	3	1	1	2	3	1.6	
H. G. 53	Essential hypertension	200	4.1	28	28	46	75	42	1034	47	65	1.010	f.t.	2	4	1.2	4.4	2.4	8.5	
M. B. 43	Essential hypertension, secondary contracted kidney	260	2.0	100	28	84	100	130	791	72	25	1.012	f.t.	2.2	(for first 5 hours)					4.2
B. A. 55	Chronic glomerulonephritis. Pyelitis contracted kidney	180	3.0	300	37	40	54	64	787	21	19	1.008	f.t.	0	0	0	0.3	0.3	1.0	
B. B. 63	Arteriosclerosis of kidney with superimposed acute inflammation	160	2.0	75	31	80	138	131	Excellent											
H. G. 39	Arteriosclerosis of kidney	222	2.7	160	28	81	100	116	672				f.t.	0	0	1	1	0	29	
B. C. 16	Acute nephritis	163	3.6	50	30	73	109	117	806	34	63	1.010	2.0	1	2	3	5	5	2.4	
E. R. 13	Valvular cardiac disease. Subacute glomerulonephritis	300	4.1	33	27	56	90	60	1016	64	80	1.020	f.t.	1.0	2.3	2.5	3.2	3.3	12.3	
Y. F. 36	Postpartum hypertension	160	4.3	37	28	57	74	42	679	67	73	1.022	f.t.	3	1.6	0.5	2.5	2.7	11.8	
J. O. 58	Diabetes insipidus	143	3.7	37	27	40	48	30	4522	80		1.003	f.t.	2.5	1.4	6.3	9	2.6	13	
S. G. 24	Diabetes mellitus	131	4.3	28	37	80	83	41	846					9	1.2	6.3		3.2	11.4	
J. S. 43	Essential hypertension (cardiac) status in kidney	248	4.1		29	53	70	64	985	51	33	1.013	Neg.	1.1	1.9	3.5	3.4	2.6	10.8	
M. B. 16	Acute glomerulonephritis	131	3.2	58	28	72	90	94	739	41	61	1.008	1.2	4	5	1.3	1.6	1.3	6.0	
N. H. 23	Acute glomerulonephritis	110	4.1	24	28	63	81	43	800	39	63	1.024	4.1	6	1.4	2.3	2.3	2.1	10.8	

\* Fermentable reducing substance

When this is done it is found that these subjects have a normal ability to excrete xylULOse as long as kidney function is intact. It is of interest that Ebstein (4), working on the metabolism of the pentose sugars, reports the case of a diabetic patient who was given 25 grams of xylULOse by mouth and after nine days was still excreting nonfermentable reducing substance in the urine. He cites this as a proof of some disturbance in the tolerance of pentose sugars in diabetics. In the protocol it is recorded that the patient shows a large quantity of albumin in the urine and we may therefore assume that the subject was retaining the xylULOse because of some damage to the kidney.

Since nonfermentable reducing substance appears in the urine of catheterized patients with normal kidneys one minute after injection of xylULOse intravenously, this pentose may find some application in surgical conditions where a quick decision as to the functional integrity of one or the other kidney is often of vital importance.

#### SUMMARY

The excretion of xylULOse is used as an index of renal function. On ingestion of 50 grams of xylULOse with limited fluid intake the intact kidney is able to concentrate the xylULOse to 2.5 per cent within 2 hours and excretes 25 per cent of the total given within 24 hours. Damaged renal function is manifested by concentrations as low as 0.1 per cent and total excretions as low as 1 gram. The curve of nonfermentable reducing substance in the blood approaches its normal value after 5 hours with intact kidney function, while in patients with impaired renal function the curve of the nonfermentable reducing substance continues upward so that values of 100 mgm per 100 cc and more are encountered. The possibility of the use of xylULOse for the determination of blood volume and in the quick diagnosis of the functional integrity of single kidneys for surgical purposes is also indicated.

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## HYPOGLYCEMIC REACTIONS FOLLOWING GLUCOSE INGESTION

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The blood sugar in normal individuals is maintained at a relatively constant level from day to day. The sugar is being continually drained from the blood stream for storage or oxidation in the tissues and is constantly being supplied to it from the liver. After taking carbohydrate food the blood in the portal vein is enriched with glucose and the liver storehouses are replenished. The blood in the systemic circulation has a greater amount of sugar for a short time after the meal than is found in the fasting condition. When insulin is given an increase both in the storage of glycogen in the liver and in the oxidation of glucose has been noted but the exact rôle played by insulin in performing these functions is still obscure. Conditions which may affect the level of the blood sugar include disturbances in the liver which may affect storage in and release of sugar from this organ and changes in the amount or quality of the internal secretion of the pancreas—insulin.

There is some variation in the normal level of the blood sugar depending on the method used. Most observers consider blood sugar readings between 80 and 120 mgm. per 100 cc. of blood as normal. Some would place the normal range between 85 and 115 mgm. In this discussion any blood sugar reading below 80 mgm. is taken as evidence of hypoglycemia. In a recent review of blood sugar methods Folin and Svedberg (1) pointed out that if plasma is used for the determination of the blood sugar instead of whole blood the discrepancies noted by many observers between the amount of fermentable sugar and the amount of reducing substances in the blood will disappear.

Many conditions have been reported where hypoglycemia was found either with or without symptoms and a brief review of some of these reports may be of interest.

1 *Fasting* Griffith (2) found levels between 28 and 54 mgm. in 9 children who were having convulsions which could be relieved temporarily by glucose ingestion. Some of these children were vomiting and some had infections but all were taking very little nourishment. He considered

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the possibility that the muscular contractions during the convulsions may have been in part responsible for depleting the blood sugar Josephs (3) reported 10 children who had fasting blood sugars between 36 and 60 mgm and he discussed fasting, infections, and constitutional factors as possible causes Talbot, Shaw, and Moriarty (4) in epileptic children who fasted noted that the blood sugar usually fell below 50 mgm sometime between the 3rd and the 6th days of the fast period They found that symptoms of a hypoglycemic nature were more common if the fluid intake was inadequate and that the younger the child the greater the fall of the blood sugar as a result of fasting Ashe, Mosenthal and Ginsberg (5) in a man with chronic nephritis who was having muscular twitchings and vomiting noted a blood sugar as low as 30 mgm They did not feel that the twitchings which were present at this time were due to the hypoglycemia alone for they were present when the blood sugar was raised to 750 mgm by the injection of glucose

2 *Physical exhaustion* The prolonged demand for glucose in continued muscular activity may lead to depletion of sugar in the body Levine, Gordon et al (6, 7) classified the condition of runners in a Marathon race on the basis of the sugar in the blood at the end of the race They found that the men who showed the most exhaustion had blood sugars below 50 mgm, those who finished in fair condition were between 60 and 70 mgm and those who were in the best condition were above 80 mgm Winans (8) studied a patient with a fasting sugar between 70 and 80 mgm who after an hour of exercise was exhausted and had a blood sugar of 68 mgm Comment has been made in the preceding section on the influence of convulsions on the blood sugar Nervous exhaustion also may play its part as pointed out by Howie and Lisherness (9), who found that 95 subjects out of 307 reported had fasting blood sugar levels below 70 mgm They point out that most of these patients showed symptoms of overwork, debilitating disease, or worry

3 *Low renal threshold for glucose* In patients with renal glycosuria a moderate depression of the blood sugar is sometimes found Fischler and Ottensooser (10) observed symptoms in phlorizinized dogs which were similar to those seen after injections of insulin

4 *Diseases of the liver* The removal of the liver in animals is followed by a sharp fall in the blood sugar (Mann and Magath, 11) Hypoglycemia has been reported in patients whose liver has been severely damaged by hydrazin, chloroform, phosphorus or white snake root Cross and Blackford (12) found blood sugars of 27 and 35 mgm with definite symptoms of hypoglycemia in a patient with severe toxic hepatitis resulting from neosalvarsan Nadler and Wolfer (13) noted low blood sugars in a patient with primary liver cell carcinoma The changes in the liver must be marked to result in hypoglycemia for it is not present in patients with cirrhosis of the liver Wakeman and Morrell (14) noted

that the blood sugar of monkeys which had been infected with yellow fever fell below normal between 24 and 36 hours before death. The level of 45 mgm was reached in their animals without symptoms of hypoglycemic reaction. They found also a marked depletion of the glycogen in the liver.

*5 Oversupply of insulin in the body* The effect of overdosage of insulin in producing hypoglycemia is so well recognized at present that it needs no special consideration.

A relative accentuation of the effect of insulin in the body has been suggested by Pettersson (15) and by Stenström (16) in patients where they noted evidence of diminished activity of other glands and blood sugars between 25 and 50 mgm. Further consideration of the interrelation of internal glandular secretions will be given in the next section. In the past four years reports of patients who have had hypoglycemia with severe symptoms which were due to tumors of the islets of the pancreas have appeared (17, 18, 19, 20, 21). Gray and Feemster (22) reported the case of a child whose mother had diabetes and who died when 3 days old, with a blood sugar of 67 mgm. At autopsy they estimated that the pancreas contained approximately 24 times the normal amount of islet tissue and that there was some hypertrophy of the medullary cells of the adrenals. They suggested that the high sugar level in the maternal blood might have been the stimulus which caused the marked development of the islets in the pancreas of the infant.

*6 Endocrine disturbance* Holman (23) reported a patient with exophthalmic goiter who lapsed into coma 24 hours after a thyroidectomy. He found a blood sugar of 48 mgm. The coma was relieved by glucose intravenously. In two other postoperative thyroid patients, the blood sugars were 78 and 80 mgm. He felt that the manipulation of the gland resulted in an excess thyroid secretion which caused a rapid utilization of the available carbohydrate with hypoglycemia.

Porges (24) reported blood sugars between 33 and 67 mgm in three patients with Addison's disease, Bernstein (25) found the range between 47 and 84 mgm in four cases and Longcope (26) between 73 and 91 mgm in five cases. Chapman (27) found only slight elevation in blood sugar after 50 grams of glucose in a patient with Addison's disease, which suggested an increased tolerance for carbohydrate in this condition.

In dogs after removal of hypophysis, Houssay and Biasotti (28) found that some animals developed convulsions and coma with the blood sugar dropping to 70 mgm and that sugar would relieve the symptoms. They noted that doses of insulin which produced no effect in controls would quickly kill the hypophysioprival animals. The tolerance to sugar was the same in both groups.

Deficiency of adrenal, pituitary or ovarian secretion has been suggested as the cause of the hypoglycemia noted in patients by Laroche,

the possibility that the muscular contractions during the convulsions may have been in part responsible for depleting the blood sugar Josephs (3) reported 10 children who had fasting blood sugars between 36 and 60 mgm and he discussed fasting, infections, and constitutional factors as possible causes Talbot, Shaw, and Moriarty (4) in epileptic children who fasted noted that the blood sugar usually fell below 50 mgm sometime between the 3rd and the 6th days of the fast period They found that symptoms of a hypoglycemic nature were more common if the fluid intake was inadequate and that the younger the child the greater the fall of the blood sugar as a result of fasting Ashe, Mosenthal and Ginsberg (5) in a man with chronic nephritis who was having muscular twitchings and vomiting noted a blood sugar as low as 30 mgm They did not feel that the twitchings which were present at this time were due to the hypoglycemia alone for they were present when the blood sugar was raised to 750 mgm by the injection of glucose

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gram of glucose per kilo of body weight with readings between 46 and 53 mgm John (44) found in a tolerance test with 100 grams of glucose that his own blood sugar fell to 45 mgm at the end of three hours and at that time he noted extreme hunger Harris (45) studied a patient who felt weak and hungry every day before lunch time and found a blood sugar of 65 mgm In a tolerance test following 100 grams of glucose the blood sugar was 67 mgm three hours after the meal Then one hour later on the way home, this patient became so faint and weak that he could hardly walk He was very hungry and his symptoms were entirely relieved after taking a meal Winans (8) reported a patient who had fasting blood sugars between 70 and 80 and who felt worse in the afternoon about three hours after lunch when the blood sugar was found to be 59 mgm

After fructose Meyer (46) noted in eleven patients that the level of the blood sugar was from 0 to 22 mgm below the fasting levels He associated this with a labile vegetative nervous system and after giving atropine to the same patients he found a rise of the blood sugar following the ingestion of fructose Cathcart and Markowitz (47) after the ingestion of 50 grams of dihydroxyacetone found blood sugars between 50 and 78 mgm which were associated with flushing, restlessness, and muscular tremors McClellan, Biasotti, and Hannon (48) after the same amount of dihydroxyacetone found similar depressions in the blood sugar but noted no symptoms

#### CASE HISTORY

In the patient to be reported we noted on three separate occasions, following the ingestion of 100 grams of glucose, symptoms which were similar to those observed in patients who have had an overdose of insulin On one occasion the blood sugar taken at this time was 40.6 mgm

The patient F R was admitted to Bellevue Hospital December 4, 1930, complaining of swelling of the legs and feet This had been present about two weeks He was 37 years of age, German by birth, and a cook by occupation The past history and family history gave no information which could be related to his present condition The patient had been out of work for about three months He had been living entirely on soup and vegetables with no meat except an occasional piece of smoked ham Two weeks previous to admission he noted that both feet and legs were swollen and that there was occasional itching and burning of the feet The swelling decreased only slightly after a night's rest in bed There had been numerous blisters on his feet and when he removed his shoes to ease the discomfort it required two to three hours for the swelling to recede sufficiently to allow him to put on his shoes again There were no symptoms particularly referable to either heart or kidney

On admission the physical examination showed an adult, white male who was not acutely ill There was marked pitting edema of both feet and ankles and in the left leg this extended almost to the knee There was no evidence of phlebitis There were no ulcerated areas on the feet at this time.

The condition was considered one of nutritional edema and he was admitted to the metabolism ward for special observations

Le'ourdy and Bussiere (29), Pettersson (15), Stenstrom (16), Wilder (30), Oppenheimer (31) and Pribram (32) Experimental evidence has been presented by Blotner and Fitz (33) to show that injections of pituitrin and adrenalin will prevent the usual fall in blood sugar when injected with insulin Also injections of adrenalin and pituitrin relieve symptoms which are associated with hypoglycemia Cammidge (34, 35) reported hypoglycemia of 70 mgm or less in 200 patients He suggested that patients with low kidney threshold for glucose and with low blood calcium may be suffering from hypoparathyroidism

The sugar level in the blood appears to be the result of a balance between the internal secretions of the thyroid, pituitary, and adrenal glands on one side and of the islets of the pancreas on the other

7 *Miscellaneous conditions* Hypoglycemia has been noted in progressive muscular dystrophy, in scleroderma (26), and in a small series of 15 patients with bronchial asthma where the blood sugar varied between 68 and 80 mgm (36) The influence of changes in the nervous system in producing hypoglycemia has been considered by Howie and Lisherness (9) and by Pemberton (37) Schmidt (38) obtained blood sugar readings between 28 and 75 mgm in 12 out of 33 patients tested one hour or less before death At autopsy these patients showed no striking pathological lesions which have been identified with the production of hypoglycemia The rôle of infection as a possible cause has been presented by Pribram (32) and by Cammidge (35)

8 *The ingestion of carbohydrate* It seems paradoxical to consider the influence of carbohydrate, which is recognized as the best means of relieving the symptoms related to hypoglycemia, among the causes of hypoglycemia It has been noted, however, by a number of observers Folin and Berglund (39) found that after giving 100 grams of glucose the blood sugar rose at first but later it fell below the fasting level with ranges between 54 and 95 mgm between two and four hours after the meal Hamman and Hirschman (40) showed that repeated tolerance tests resulted in smaller elevations of the blood sugar, and they point out that the evidence of an increased tolerance for carbohydrate is present when there is only a slight rise after the glucose meal In this group they noted a greater tendency toward hypoglycemia after the meal In a patient with renal glycosuria Gibson and Larimer (41) found a fasting blood sugar of 58 mgm and after 50 grams of glucose the level was at 40 mgm three and one half hours later and the patient had mild symptoms Then they gave another 50 grams of glucose and three and one half hours later it was 58 mgm and slight symptoms were noted again In three patients with fasting blood sugars between 100 and 105 mgm, Foster (42) found the level between 61 and 81 mgm two hours after giving 100 grams of glucose Stenstrom (43) noted in four patients with fasting blood sugars between 76 and 95 mgm hypoglycemia about two hours after they had taken 1

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The laboratory data showed that his urine was normal and his blood Wassermann negative. The findings for his blood chemistry on December 11, 1930 are presented in Table I.

TABLE I

*The blood chemistry findings of F. R. on December 11, 1930, one week after admission to the hospital*

Nonprotein nitrogen	45.0 mgm per 100 cc
Blood sugar	81.0 mgm per 100 cc
Blood chlorides	480 mgm per 100 cc
Blood phosphorus	5.0 mgm per 100 cc
Blood calcium	12.4 mgm per 100 cc
CO <sub>2</sub> combining power	57.6 volumes per cent
Total serum protein	6.28 grams per 100 cc
Albumin protein	3.53 grams per 100 cc
Globulin protein	2.75 grams per 100 cc

In the ward he was placed on a diet of 3000 calories and his edema entirely disappeared at the end of 48 hours. He was observed for a period of two months with diets containing both large and small amounts of carbohydrate. Details of the food taken and the analyses of the urine for the period of observation are presented in Table II.

TABLE II

*Data of food ingested and the analyses of urine given as the average daily findings for each period of the observation*

Period number	Number of days	Weight	Food					Urine			
			Calories	Protein	Fat	Carbohydrate	N <sub>2</sub>	N <sub>2</sub>	Acetone bodies	Acidity	Volume
		kgm	calories	grams	grams	grams	grams	grams	grams	cc O I N	cc
I	5	55.7	2800	80	134	300	12.8	12.5		339	1632
II	11	56.4	2976	79	133	344	12.6	9.8		319	1900
III	11	56.2	3000	80	133	350	12.8	9.1		310	1656
IV	7	56.7	2911	75	128	344	12.0	8.4		156	1310
V	10	57.1	2962	76	131	350	12.2	8.6		249	1376
VI	9	57.4	2922	78	237	90	12.5	9.5	0.86*	352	1484
VII	7	56.6	2878	78	264	25	12.5	11.1	3.84	529	1411
VIII	6	57.5	2847	73	127	333	11.7	8.4	0.80† 0.15 negative	224	1533

\* First day omitted

† First 2 days of period

The special studies carried out included observations of his respiratory metabolism in the calorimeter following the ingestion of 100 grams of glucose. In each of the three observations made he developed symptoms about four and one-half hours after the ingestion of the glucose, which made it necessary on each occasion to terminate the observation. It was not until the second occurrence of the symptoms that their similarity to those following the injection of insulin was considered. For that reason actual observation of the blood sugar was made only once, when it was found to be 40.6 mgm.

The finding of this low level of blood sugar suggested the possibility that this man might be suffering from a mild degree of hyperinsulinism. A single blood sugar time curve was obtained after giving him 100 grams of glucose. Mild symptoms including slight dizziness, flushing of the face and moisture on the skin were noted three and one half hours after giving the glucose when his blood sugar was 60.5 mgm. He left the hospital against our advice on February 12, 1931, with a diagnosis of alimentary hyperinsulinism.

## DISCUSSION

### *A The effect of the ingestion of glucose on the blood sugar*

As has been pointed out in the review of previous work on this subject, the response of the body to the ingestion of glucose depends in a large part on the previous diet which the patient has been receiving. The relatively high levels to which the blood sugar rises in the tolerance test with patients in whom the carbohydrate in the diet has been markedly restricted is well established by the work of Malmros (49) and others. Tolstoi (50) found that the Arctic explorers who had lived on an exclusive meat diet for one year showed high levels of the sugar in the blood during tolerance tests following 100 grams of glucose.

We have made a few observations of the blood sugar tolerance curves of five subjects, some of whom were receiving high carbohydrate diets and others of whom received only 25 grams of carbohydrate per day. While our series is not of sufficient size to warrant statistical consideration, still the curves obtained seem worth reporting. After the fasting blood was obtained the men were given 100 grams of glucose in the form of 104 grams commercial glucose, 100 grams orange juice and 200 cc. of water. The samples of blood were obtained at 45, 90, 150, 210 and 270 minutes after the ingestion of the glucose. The analyses of sugar in the blood were made in duplicate samples by the modified Hagedorn Jensen method (51). In a few of the tests the analyses were checked by the Folin Wu method (52). Curves showing the results of these tolerance tests and the composite curve for each group are presented in Chart 1.

There was some scatter in the observations of the men who had been receiving in their diets large amounts of carbohydrate. In two subjects, F. R. and W. C., scarcely any elevation in the blood sugar was noted, in the subject E. A. following a period on a low carbohydrate diet, the highest level of the blood sugar was found. It should be pointed out that this subject received only 45 grams of protein in his diet when the test was made, while the other two subjects were receiving 90 grams per day. This may in part account for the higher level observed in this subject. Another point of particular interest was the finding that after four and one-half hours following the ingestion of glucose the level of the blood sugar in all subjects was at almost the same level regardless of whether they had been receiving high or low carbohydrate diets. No



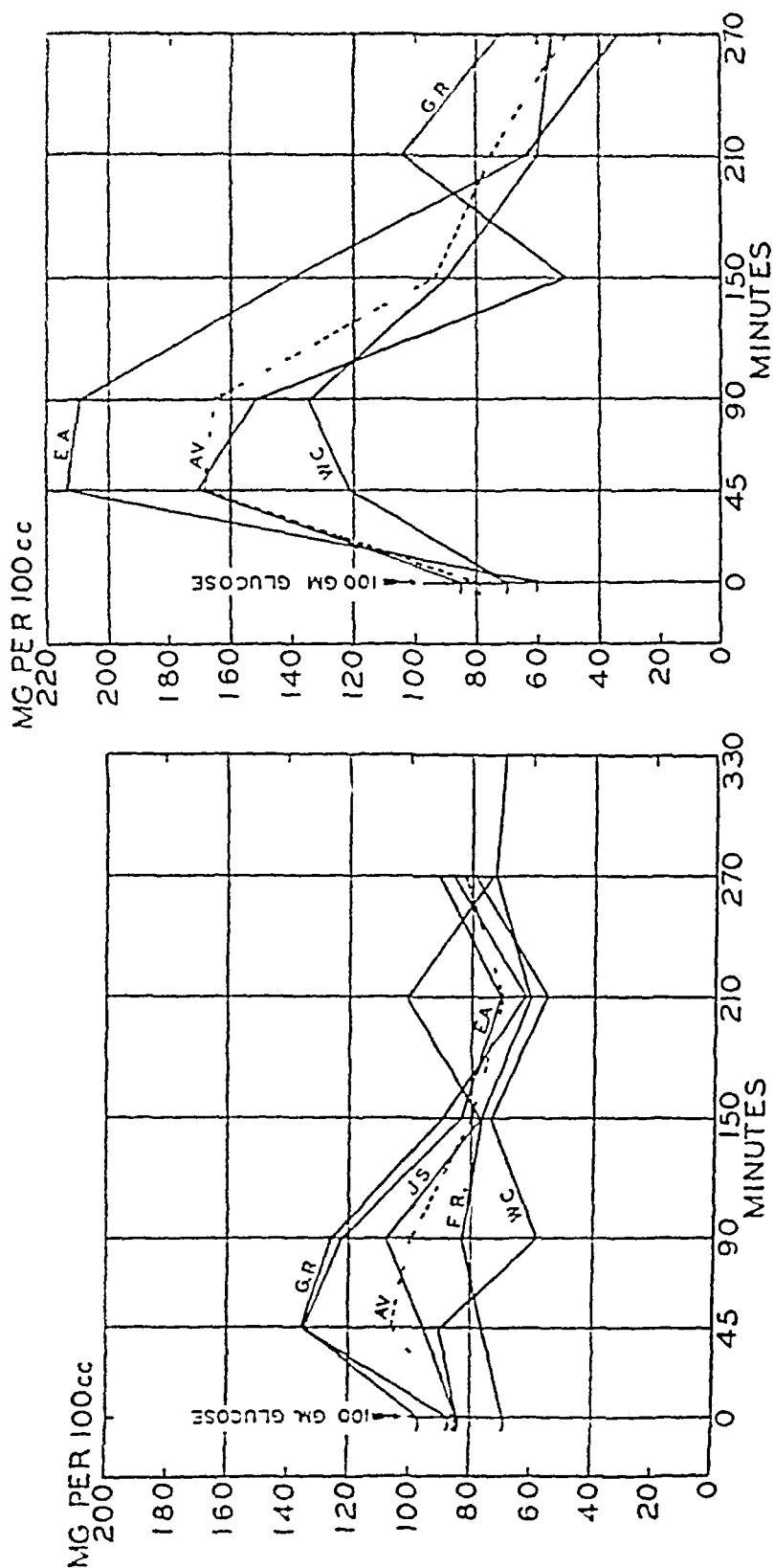


CHART 1 THE BLOOD SUGAR TIME CURVES OF FIVE SUBJECTS WITH REFERENCE TO THE NATURE OF THE PREVIOUS DIET  
 The first group were made when the patients were receiving 350 to 400 grams of carbohydrate daily in the diet, and the second group when they were receiving only 25 grams daily. The broken line is the composite curve for each group.

symptoms referable to hypoglycemia were noted in any subject except F R, the one whose special observations are being presented in this paper

### *B Relation of level of the blood sugar to the occurrence of symptoms*

Most observers consider that a blood sugar of 80 mgm is the lower limit of the normal range. Below this point blood sugars ranging between 60 and 80 represent a mild hypoglycemia and are usually not associated with definite symptoms. When the level is below 60 some symptoms are usually expected. In our subjects levels below 60 occurred four times but in only one instance were any symptoms noted. One observation of the subject F R, who developed symptoms of hypoglycemic reaction while in the calorimeter, showed at that time a level of 40.6 mgm. In the subject E A a level of 34.0 mgm was reached in one tolerance test without the development of symptoms. It would appear from our observations that the development of symptoms must be associated with some other factor than just the level of sugar in the blood alone. Inasmuch as the symptoms are similar to those seen in overactivity of the sympathetic nervous system, it seems possible that the development of symptoms may be in some way associated with different degrees of sensitivity in this system.

### *C The respiratory metabolism*

The basal metabolism of the subject F R as determined in the Sage calorimeter was 62 calories per hour, which represented 97 per cent of the average normal metabolism determined by the Aub-DuBois standards (53). The metabolism following the glucose meals which resulted in the hypoglycemic reaction described above is presented in Chart 2. In each observation the record covers the second, third, and fourth hours following the ingestion of the glucose. The reaction in each case occurred early in the fifth hour so that no accurate results were obtained of his metabolism at that time. The first two observations were made while he was receiving a diet containing 80 grams protein, 133 grams fat and 350 grams carbohydrate. The last observation followed a period when he received only 25 grams carbohydrate in his diet. His response to the ingestion of glucose under these conditions showed two striking differences. First, a marked rise in the respiratory quotient to nearly 1.00, which shows that his energy was being derived almost exclusively from carbohydrate in the observations following the high carbohydrate diet. Second, the absence of any marked rise in the respiratory quotient in the observation following the low carbohydrate diet, which indicates that he was using only slightly greater amounts of carbohydrate in this observation than he did when in basal condition. The hatched portion at the bottom of each column represents the calories derived from the protein

metabolized, the clear portion of the column the calories derived from fat and the vertical lining the portion from carbohydrate

The disposal of the 100 grams of carbohydrate in this subject under the two conditions studied differs materially. In the three hours observed in the first two observations 42 grams of carbohydrate were ac-

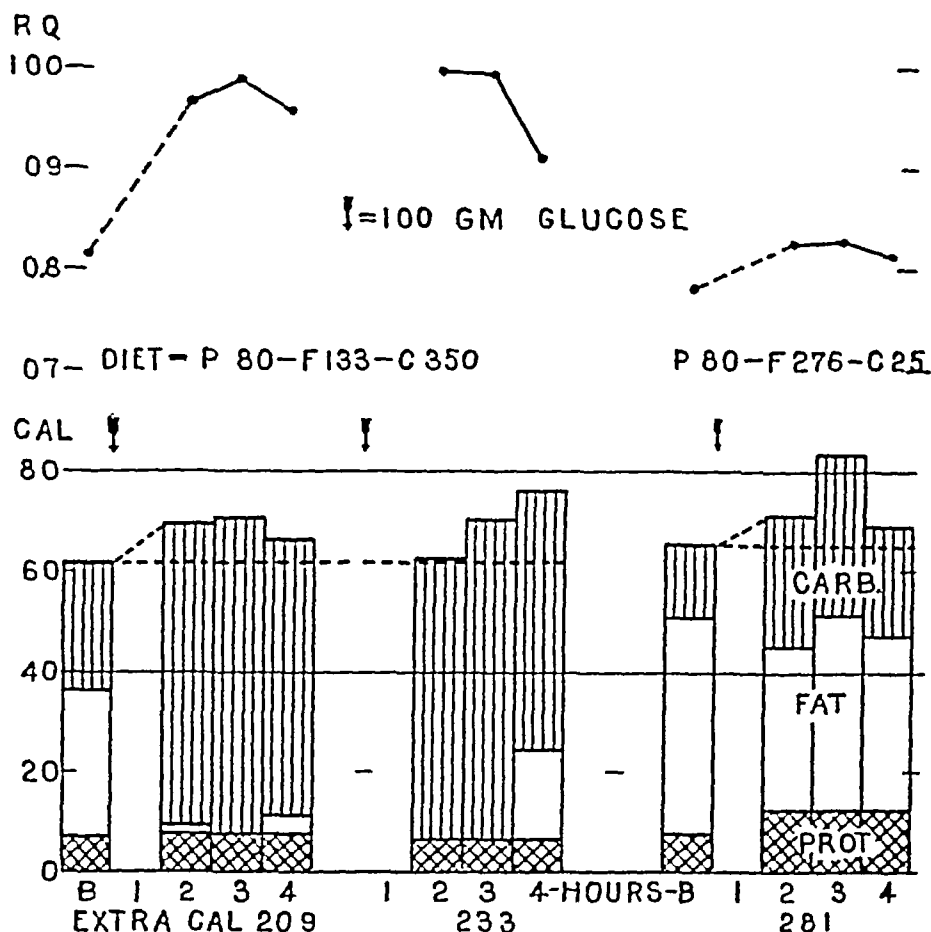


CHART 2 THE RESPIRATORY METABOLISM OF F R IN BASAL CONDITION AND AFTER GLUCOSE INGESTION

The nature of the previous diet and the excess metabolism is indicated. The height of the columns shows the total metabolism per hour and the calories resulting from the metabolism of protein are represented by the hatched portion at the bottom of the column, those from fat by the clear space and those from carbohydrate by the vertical lined portion at the top. The curves show the changes in the level of the respiratory quotient.

tually oxidized while in the last observation only 23 grams were metabolized. At no time did we find more than a trace of glucose in his urine. It appears then that when there was an adequate supply of glycogen in the body, the subject actually metabolized a much greater portion of the ingested glucose than he did when the glycogen stores were depleted by a



metabolized, the lower portion of the column the calories derived from fat and the vertical lining the portion from carbohydrate.

The disposal of the 100 grams of carbohydrate in this subject under the two conditions studied differs materially. In the three hours observed in the first two observations 12 grams of carbohydrate were ac-

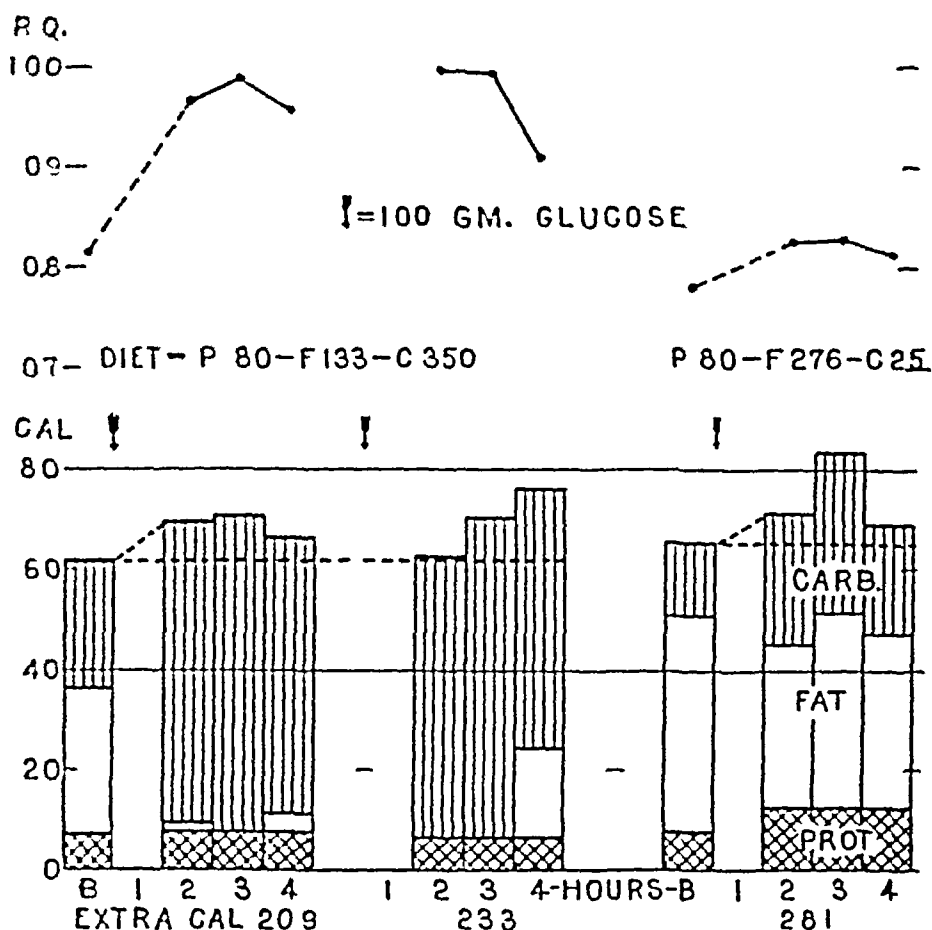


CHART 2 THE RESPIRATORY METABOLISM OF F. R. IN BASAL CONDITION AND AFTER GLUCOSE INGESTION

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# THE COPPER AND NON-HEMOGLOBINOUS IRON CONTENTS OF THE BLOOD SERUM IN DISEASE<sup>1</sup>

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This is a report of an investigation into the extent to which the copper and non hemoglobinous iron levels of the blood serum are shifted in disease, and the extent to which the shifted levels may reflect underlying disturbances of tissue respiration and metabolism.

The levels of copper and non hemoglobinous iron in the blood serums of fasting individuals are determined by balances between the rates at which the metals are drawn or excreted from the cells and the rates at which they are excreted from the body. In the absence of changes in the rates at which the metals are excreted from the body, increased levels in the serum signify decreased levels within the cells, and vice versa.

Increases in the copper content of normally respiring cells would appear to be accompanied by a reduction in the intensity of their metabolism. The respiration of the unripe *Arbacia* egg decreases progressively as the content of copper increases. Following fertilization, the copper content becomes reduced, as the result of the excretion of a "copper-avid" substance into the supporting medium, and the rate of respiration rises (1). Additions of copper to cultures of baker's yeast are followed by proportionate decreases in the rates of respiration and glycolysis (2). Concentrations of copper equivalent to those present in human serums are not only partially inhibitory to respiration and glycolysis but also to proteolysis (3) (4) and lipolysis (5) (6) as encountered in the cells which make up mammalian tissue.

Increases in the iron content of living cells would appear to be followed by an augmentation of metabolism. Additions of iron to cultures of the anaerobic *Cl. sporogenes* are followed by increases in metabolism of such intensity as to lead to the disintegration of the organisms (7). Comparable additions to cultures of baker's yeast are without effect (2). Aerobic cells appear to have a mechanism for holding the iron level within a tolerable range, presumably through the removal by oxidation and excretion of all but a minimal fraction of the catalytic iron intake. Impairment of this mechanism through a restriction of the oxygen intake may, in healthy

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animals be followed by a temporary stimulation of metabolism, reflected in an increased rate of production of red blood corpuscles and an augmentation of functional activity in general (8). In tumor cells, where the oxidation mechanisms may be defective (9), ferrous iron may tend to accumulate to a level sufficiently high to initiate the increased rate of growth, death, and autolysis which is associated with the development of tumor tissue.

The first determinations of the copper and non-hemoglobinous iron contents of human serum were published by Warburg and Krebs in 1927 (10). The analyses were made by a catalytic method, developed by Warburg (11), which appears to give accurate values in the hands of persons trained in its use. The series was extended to a total of eighty-nine serums by Krebs in 1928 (12), the added analyses being restricted to determinations of copper content. The serums of ten normal individuals were examined. Separation of the figures reported into values characterizing men and values characterizing women indicates an average copper level for the former of 0.82 microgram and for the latter of 0.98 microgram per cc. The average copper level for twelve persons with pulmonary tuberculosis was 1.55 microgram per cc, for thirteen women in the final months of pregnancy, 2.07. Analyses of the serum in other conditions also were reported, the values being, on the whole, comparable to those reported herewith.

Analyses of whole blood for copper have been made by Schindel (13), using the Schonheimer-Oshima modification (14) of the Spacu-Biazzo method (15), a method which is not practicable for general use because of the large amount of sample required. The values appear to be high. They corroborate the distribution of copper between the maternal and fetal blood reported by ourselves, and the distribution of copper between the plasma and corpuscles reported by Flinn and Inouye (16). Additional analyses of human blood for copper have been reported by Schonheimer and Oshima (14), Kleinmann and Klinke (17), Herkel (18), and Grendel (19).

Analyses of the bloods of other species are reported by McHargue (20), the pioneer in the field, and by Warburg and Krebs (10), Abderhalden and Moller (21), and Grendel (19). The values for the copper content of horse serum reported by Elvehjem, Steenbock and Hart (22) are out of line with the results obtained by ourselves and others. Their method of ashing is possibly not adapted to serum because of the risk of volatilizing copper chloride.

The values reported by Warburg and Krebs for the non-hemoglobinous iron contents of human serums would, perhaps, have been in closer agreement with those reported herewith had more determinations been made. The values reported by Riecker (23) (24), using a method similar to our own, are, singularly enough, approximately ten times too high.

The rise of the iron level in untreated pernicious anemia and its correction following liver therapy, as observed by Riecker, are confirmed in this report, as are his observations of a lowered iron level in anemia secondary to hemorrhage. The latter observation has been reported also by Fontès and Thivolle (25)

Values for the iron content of the serums of species other than man have been reported by Barkan (26), Starkenstein and Weden (27), Warburg and Krebs (10), Henriques and Roche (28), Abderhalden and Möller (21), and Fontès and Thivolle (25)

#### METHODS

The following procedures permit the determination of the copper and non hemoglobinous iron contents of ten cc. of fresh blood serum with an accuracy of  $\pm$  ten per cent and with the time, apparatus, and technic at the disposal of the ordinary clinical laboratory

Ten cc. of the serum to be examined are measured into a carefully cleansed well seasoned 50 cc centrifuge tube. An equal volume of a 20 per cent solution of redistilled trichloroacetic acid is added and the well suspended mixture permitted to stand for 2 hours, with occasional resuspension, before centrifugation for 30 minutes at a speed of 1500 rotations per minute. Two portions 4 cc and 3 cc. of the clear supernatant liquid are transferred to 125 cc. glass stoppered Erlenmeyer flasks for copper analysis. Two further portions of 3 cc and 2 cc are transferred to similar flasks for iron analysis. Equivalent volumes of 10 per cent trichloroacetic acid are taken for use as blanks.

*Copper analysis* Each aliquot is diluted with glass-distilled water, together with the blank and, occasionally, a reference control, to a volume of 9 cc. One drop of 1 per cent phenolphthalein is added, followed by a sufficient volume of a saturated solution of metal free sodium hydroxide to produce a stable red color. The color is discharged by the addition of an excess of 3 drops of glacial acetic acid. Approximately 0.2 cc. of pyridine<sup>1</sup> are added and 1 cc. of a 1 per cent solution of Eastman's sodium diethyl dithio carbamate which is free of sediment.<sup>2</sup> The mixtures are permitted to stand for 1 hour, when exactly 2 cc. quantities of amyl alcohol are added. After vigorous shaking, and standing for the 2 to 3 minutes required for the separation of the water and alcohol layers, the latter are transferred to small test tubes with Pasteur pipettes equipped with small rubber suction bulbs. In the unusual event that the iron content of the sample is greatly in excess of the copper content, the alcohol extracts may have a transient brown black tinge which must be allowed to fade before making the colorimetric comparison with the standards. The extracts are ordinarily a stable, clear yellow in color. The color of the standards is developed at the same time and in the same way as that of the unknown supernatant. It is convenient to use standards containing 1.5 to 2.0 micrograms of copper per 2 cc. of alcohol extract, and to prepare them from a solution of copper sulphate in 0.01 N sulphuric acid which contains 5 micrograms of copper per cc.

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<sup>1</sup> The pyridine does not enter into the composition of the color complex

<sup>2</sup> The use of this reagent for the detection and estimation of copper was first recommended by Callan and Henderson (29). The procedure for iron analysis is adapted from a method proposed by R. P. Kennedy (30)

*Iron analysis.* No water is added. The stoppers of the flasks are removed, 1 cc. of redistilled sulphuric acid and 3 drops of perhydrol are added, and the mixtures kept on an asbestos covered hot plate, at a temperature sufficient to maintain 50 cc. of water in a similar container at a temperature of 75 to 85° C., until incipient charring is manifested by a distinct yellow or brown coloration in the digest. A sufficient volume of a 0.05 N solution of iron free potassium permanganate is added to the partly cooled digests to accomplish their decolorization. Not more than 2 to 3 drops should be required. Approximately 15 cc. of glass-distilled water are added and the heating resumed for 30 minutes. The contents of the flasks are cooled, the stoppers replaced, and 2 more drops of permanganate solution added. Exactly 2 cc. of amyl alcohol are added while the permanganate color still persists and are followed at once by the addition of 10 cc. of a 20 per cent solution of iron free potassium thiocyanate. After thorough shaking, the flasks are allowed to stand for 2 to 3 minutes. The alcohol layers are removed, and the colors compared with those of standards, as described in the section on copper analysis. The colors of the standards should be closely equivalent to those of the unknowns with which they are to be compared and readings should be made rapidly before appreciable fading can occur. It is convenient to have ready a series of standards containing 0.5, 1.5, and 2.5 micrograms of iron per 2 cc. of alcohol extract, and to prepare them from a solution of ferrous ammonium sulphate in 0.01 N sulphuric acid which contains 5 micrograms of iron per cc. The iron standards contain 1 cc. of sulphuric acid in the 15 cc. of their total volume but are not heated and contain no perhydrol or trichloroacetic acid.

The colorimetric comparisons may be made, with accuracy, in inexpensive 6 to 8 x 75 mm. tubes with optically flat bottoms. The tubes are covered with black paper and the contents compared by looking through at a white porcelain surface, well illuminated by reflected sunlight. The lengths of the liquid columns are varied with the aid of the Pasteur pipettes used for the isolation of the alcohol extracts and the readings made by application of a small straight-edge ruler.

The copper and iron blanks should not exceed 0.4 microgram in total amount and must be determined with the same care as the metal contents of the samples themselves.

The amounts of copper and non-hemoglobinous iron in 1 cc. of serum are, within the limits of error inherent in the method, twice those present in the trichloroacetic acid extract used for analysis.

Copper, in amounts appreciably in excess of those encountered in human serum, tends to interfere with the redness of the color normally produced by ferric ion with thiocyanate. Iron does not seriously interfere with the determination of copper, by the procedure described, even when present in amounts considerably in excess of those found in human serum. The presence of a trace of hemoglobin in the serum does not affect the recovery of either copper or iron.

### *Normals*

Table 1 presents the distribution of copper and non-hemoglobinous iron in the blood serums of a group of hospital employees, apparently well and actively engaged in work. The blood was obtained in carefully cleansed and dried containers before breakfast and kept in the refrigerator for three to six hours before separation of the serum by centrifugation. The analyses were carried out as has been described.

TABLE 1

*The copper and non hemoglobinous iron contents of the blood serums of a group of twenty eight 'normal' adults*

Distribution	Serum		Per cent of serum in blood	Serum		Ratio of copper to iron
	Copper	Iron		Copper	Iron	
	micrograms per cc. of serum	micrograms per cc. of serum	per cent	micrograms per cc. of blood	micrograms per cc. of blood	
Men						
3	0.79 <sup>bt</sup>	1.45 <sup>bt</sup>	47 <sup>bt</sup>	0.37 <sup>bt</sup>	0.68 <sup>bt</sup>	0.5 1 <sup>bt</sup>
1	81	1.38 <sup>ft</sup>	43	.35	.59 <sup>ft</sup>	6 1 <sup>ft</sup>
3	79	1.15	46	.36	.51	7 1
2	73	90	43	.31	.38	8 1
2	1.06 <sup>ht</sup>	1.29 <sup>ht</sup>	44 <sup>ht</sup>	.47 <sup>ht</sup>	.57 <sup>ht</sup>	8 1 <sup>ht</sup>
2	87	87	45	.39	.39	1.0 1
1	1.08 <sup>t</sup>	83	49 <sup>t</sup>	.53 <sup>t</sup>	.41	1.3 1 <sup>t</sup>
Women						
2	.79 <sup>bt</sup>	1.41 <sup>bt</sup>	51 <sup>bt</sup>	.40 <sup>bt</sup>	.72 <sup>bt</sup>	6 1 <sup>bt</sup>
1	81 <sup>lt</sup>	1.04 <sup>lt</sup>	50 <sup>lt</sup>	.41 <sup>lt</sup>	.52 <sup>lt</sup>	8 1 <sup>lt</sup>
3	95	92	52	.49	.48	1.0 1
2	1.06 <sup>at</sup>	.94 <sup>at</sup>	50 <sup>at</sup>	.53 <sup>at</sup>	.47 <sup>at</sup>	1.1 1 <sup>at</sup>
3	90	77	54	.49	.42	1.2 1
3	90	62	57	.51	.35	1.5 1
Averages						
Men (8)	0.80±.8	1.00±.15	45±.2	0.35±.4	0.44±.7	0.8±.2 1
Women (9)	.92±.3	.77±.15	54±.3	.50±.1	.42±.7	1.2±.2 1

a age more than 50 yrs, b bilirubinemia, f fasting interval more than 12 hours, h later found to have a high basal metabolic rate, l later found to have a low basal metabolic rate, t not considered in the averages

The presence of bilirubin was apparent in several of the serums examined, through the bluish green color of the products of oxidation carried into the trichloroacetic acid precipitates during the preparation of the samples for analysis. These serums appeared to contain more iron than serums giving a colorless precipitate with trichloroacetic acid. They were not considered in the computation of the averages.

Others of the group could not be considered in the averages for the reasons indicated in the footnotes appended to Table 1.

The majority of the men had lower, and more variable, copper levels, and higher iron levels than were found in the majority of the women. The women's blood contained more serum than the men's blood. The differences in the copper levels of the two sexes became accentuated, and the differences in the iron levels minimized, when this factor was taken into account. The copper-iron ratio characteristic of the majority of the women was approximately 50 per cent higher than that characteristic of the majority of the men.



*Iron analysis.* No water is added. The stoppers of the flasks are removed, 1 cc of reconstituted sulphuric acid and 3 drops of perhydrol are added, and the mixtures kept on an asbes or covered hot plate, at a temperature sufficient to raise up 50 cc of water in a similar container at a temperature of 75 to 85° C., until incipient charring is manifested by a distinct yellow or brown coloration in the digest. A sufficient volume of a 0.05 N solution of iron free potassium permanganate is added to the partly cooled digests to accomplish their decolorization. Not more than 2 to 3 drops should be required. Approximately 15 cc of glass-distilled water are added and the heating resumed for 30 minutes. The contents of the flasks are cooled, the stoppers replaced, and 2 more drops of permanganate solution added. Exactly 2 cc of amyl alcohol are added while the permanganate color still persists and are followed at once by the addition of 10 cc of a 20 per cent solution of iron free potassium thiocyanate. After thorough shaking, the flasks are allowed to stand for 2 to 3 minutes. The alcohol layers are removed, and the colors compared with those of standards, as described in the section on copper analysis. The colors of the standards should be closely equivalent to those of the unknowns with which they are to be compared and readings should be made rapidly before appreciable fading can occur. It is convenient to have ready a series of standards containing 0.5, 1.5, and 2.5 micrograms of iron per 2 cc of alcohol extract, and to prepare them from a solution of ferrous ammonium sulphate in 0.01 N sulphuric acid which contains 5 micrograms of iron per cc. The iron standards contain 1 cc of sulphuric acid in the 15 cc of their total volume but are not heated and contain no perhydrol or trichloroacetic acid.

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The increased levels accompanying incipient starvation may mirror the progressive depletion, in the cell nutrient, of substances capable of protecting the iron-carrying catalysts from oxidation. Substances of the iron sparing type may be essential constituents of liver and other extracts effective for the correction of pernicious anemia. In vitro, additions of liver extract to cultures of *Cl. welchii* are as effective in stimulating the production of hemotoxin as further additions of ferrous iron (31). Pernicious anemia may be, in part, the end result of a chronic deficiency of substances capable of curbing the wasteful burning-out of the catalysts of cellular metabolism.

Brugsch and Irger (32) report an apparently quantitative relationship between the amounts of iron and bilirubin which are excreted in the bile of dogs. No parallel, inflexible relationship appears to exist in the serum of man, since we have found increased concentrations of the pigment in the serums of several persons with normal or abnormally low iron levels. Uncomplicated bilirubinemia has been accompanied by a high serum iron level without exception, as has icterus resulting from cirrhosis of the liver.

*Conditions in which the iron content of the serum is decreased, the copper content remaining within the normal range*

The non hemoglobinous iron content of the serum is decreased, without significant accompanying increase or decrease in the copper level, in conditions analogous to carbon monoxide poisoning, associated with a depletion in the oxygen carrying power of the blood, and in conditions analogous to barbitol poisoning, associated with a depression of the rate of intracellular oxidation.

The rabbits described in Table 3 were, for the most part, afflicted with parasitic infestations of the intestinal tract and other complications which prevented the establishment of true rabbit normals. It seemed impossible to purchase animals in perfect health. For this reason, emphasis is placed on the per cent change in the copper and iron levels, rather than on the absolute values obtained.

The test bleedings of the rabbits were followed by a fairly uniform drop of 10 to 15 per cent in the hemoglobin level, a parallel drop in the non-hemoglobinous iron level (in the absence of complications), and a 20 to 30 per cent drop in the copper level.

No further drop in the copper level was observed following the institution of bi-diurnal bleedings or of poisoning with carbon monoxide or barbitol.

The decreased iron levels observed in the serums of the rabbits exposed to carbon monoxide were due, specifically, to the depleting effect of that gas upon the oxygen-carrying capacity of the blood. Control rabbits, kept in chambers ventilated in an identical manner but free of

carbon monoxide showed no parallel changes excepting for the initial drop of the copper and iron levels which follows the institution of weekly bleedings. Other rabbits, subjected to a depletion in blood oxygen carrying capacity comparable to that obtained with the carbon monoxide, but as the result of bi-diurnal bleedings, responded with comparable decreases in the non-hemoglobinous iron content of their serum.

The metal levels in the instances of secondary anemia, in man, which have come to our attention have been influenced principally by the complications present.

It is an interesting commentary on the faithfulness with which both the normal and the diseased organisms tend to maintain a characteristic

TABLE 3

*The copper and non hemoglobinous iron contents of the serum in conditions associated with a depression in the basal metabolic rate*

Description		Serum		Ratio of copper to iron
		Copper	Iron	
		<i>micrograms per cc.</i>	<i>micrograms per cc.</i>	
Rabbits subjected to a 10-15 per cent depletion in blood O <sub>2</sub> -carrying capacity as the result of weekly bleedings *	Before	0.50	1.67	0.3 1
	After	.35	1.45	24 1
	<i>Change, per cent</i>	-30	-15	-20
Rabbits subjected to a 25-30 per cent further decrease in blood O <sub>2</sub> -carrying capacity as the result of exposure to carbon monoxide *	Before	72	1.40	5 1
	After	47	42	11 1
	<i>Change, per cent</i>	-35	-70	+120
Rabbits subjected to weekly bleedings and, in addition, injected with sub-lethal doses of barbitol †	Before	64	1.37	5 1
	After	52	.52	10 1
	<i>Change, per cent</i>	-20	-60	+100
	After recovery	53	1.42	4 1
Rabbits subjected to a 30 per cent depletion in blood O <sub>2</sub> -carrying capacity as the result of bi-diurnal bleedings ‡	Before	72	1.23	6 1
	After	.54	48	11 1
	<i>Change, per cent</i>	-25	-60	+80
Men				
Normal		80±8	1.00±15	0.8±2 1
Barbitol poisoning		91	27	3.7 1
	<i>Divergence, per cent</i>	+15	-75	+360
Dwarf, basal metabolic rate -26		95	42	2.3 1
	<i>Divergence, per cent</i>	+20	-60	+190

TABLE 3 (continued)

Description	Serum		Ratio of copper to iron
	Copper	Iron	
	<i>micrograms per cc.</i>	<i>micrograms per cc.</i>	
Women Normal	92±3	77±15	1.2±2.1
Menorrhagia basal metabolic rate -16†	85	15	5.7.1
Divergence per cent	-10	-80	+370
Pituitary ovarian dysfunction basal metabolic rate +4	1.24	.33	3.8.1
Divergence per cent	+35	-60	+220
Barbital poisoning	1.42	.39	3.6.1
Divergence per cent	—	-50	—
Low basal metabolic rate (-27) of undetermined origin	79	40	2.0.1
Divergence per cent	-15	-50	+65
Hypothyroidism no medication basal metabolic rate -27	77	89	9.1
Divergence per cent	-20	+15	-25
Hypothyroidism receiving thyroid basal metabolic rate -4	1.50	90	1.6.1
Divergence per cent	+60	+15	+35
Nephritis without edema ‡	96	97	1.0.1
Nephritis with tendency to edema ‡	75	41	1.8.1
Addison's disease cyanosis starvation basal metabolic rate -30	1.61	70	2.3.1
Polycythemia vera cyanosis basal metabolic rate -4	64	99	7.1

\* Average of 3 consistent series

† Average of 4

‡ Average of 2

copper iron picture that while differences of more than 100 per cent were found in the initial copper and iron levels of the different rabbits used in the experiments reported in Table 3, those same differences were again established upon recovery from the experiences to which they were subjected. Recovery was, of course, to the status of rabbits used for weekly bleedings and not to the status of unused rabbits.

The decreased serum iron levels observed in the rabbits injected with

carbon monoxide, showed no parallel changes excepting for the initial drop of the copper and iron levels which follows the institution of weekly bleedings. Other rabbits subjected to a depletion in blood oxygen-carrying capacity comparable to that obtained with the carbon monoxide, but as the result of bi-diurnal bleedings, responded with comparable decreases in the non-hemoglobinous iron content of their serums.

The metal levels in the instances of secondary anemia, in man, which have come to our attention have been influenced principally by the complications present.

It is an interesting commentary on the faithfulness with which both the normal and the diseased organisms tend to maintain a characteristic

TABLE 3

*The copper and non-hemoglobinous iron contents of the serum in conditions associated with a depression in the basal metabolic rate*

Description	Serum		Ratio of copper to iron
	Copper	Iron	
	micrograms per cc.	micrograms per cc.	
Rabbits subjected to a 10-15 per cent depletion in blood O <sub>2</sub> -carrying capacity as the result of weekly bleedings *	Before After Change, per cent	1.67 1.45 -15	0.3 1 24 1 -20
Rabbits subjected to a 25-30 per cent further decrease in blood O <sub>2</sub> -carrying capacity as the result of exposure to carbon monoxide *	Before After Change, per cent	1.40 .42 -70	.5 1 11 1 +120
Rabbits subjected to weekly bleedings and, in addition, injected with sub-lethal doses of barbitol †	Before After Change, per cent After recovery	1.37 .52 -60 1.42	5 1 10 1 +100 4 1
Rabbits subjected to a 30 per cent depletion in blood O <sub>2</sub> -carrying capacity as the result of bi-diurnal bleedings †	Before After Change, per cent	1.23 .48 -60	6 1 11 1 +80
Men			
Normal	80±8	100±15	0.8±2 1
Barbitol poisoning	91 +15	.27 -75	3.7 1 +360
Dwarf, basal metabolic rate -26	95 +20	42 -60	2.3 1 +190
	Discrepancy, per cent		

TABLE 3 (continued)

Description	Serum		Ratio of copper to iron
	Copper	Iron	
	micrograms per cc.	micrograms per cc.	
Women			
Normal	92±3	77±15	1.2±2.1
Menorrhagia basal metabolic rate -16†	85	15	5.7.1
Divergence per cent	-10	-80	+370
Pituitary ovarian dysfunction basal metabolic rate +4	1.24	33	3.8.1
Divergence per cent	+35	-60	+220
Barbital poisoning	1.42	.39	3.6.1
Divergence, per cent	—	-50	—
Low basal metabolic rate (-27) of un- determined origin	79	40	2.0.1
Divergence per cent	-15	-50	+65
Hypothyroidism no medication basal metabolic rate -27	77	89	9.1
Divergence per cent	-20	+15	-25
Hypothyroidism receiv- ing thyroid basal metabolic rate -4	1.50	90	1.6.1
Divergence per cent	+60	+15	+35
Nephritis without edema †	96	97	1.0.1
Nephritis with tendency to edema †	75	41	1.8.1
Addison's disease cyanosis starvation basal metabolic rate -30	1.61	70	2.3.1
Polycythemia vera cyanosis basal metabolic rate -4	64	99	7.1

\* Average of 3 consistent series

† Average of 4

‡ Average of 2

copper iron picture that while differences of more than 100 per cent were found in the initial copper and iron levels of the different rabbits used in the experiments reported in Table 3 those same differences were again established upon recovery from the experiences to which they were subjected. Recovery was of course, to the status of rabbits used for weekly bleedings and not to the status of unused rabbits.

The decreased serum iron levels observed in the rabbits injected with

sub-lethal doses of barbitol were found also in two persons suffering with barbitol poisoning. Analogous decreases were observed in two instances of menorrhagia, in one of dwarfism, and in one of combined pituitary and ovarian dysfunction.

The basal metabolic rate is reported to be lower in nephritis with edema than in nephritis without edema (33). The metal levels in the patients with Addison's disease and polycythemia vera were affected by the complications present.

The copper level in the single instance of untreated hypothyroidism available was slightly below the levels normally observed in women. The iron level was not depressed, differentiating the condition from those in which the basal metabolic rate was depressed as the result of pituitary and ovarian dysfunction.

Increases in the copper content of the serum, indicative as they are of a depletion of the copper content of the tissues and a consequent decreased extent of inhibition of cellular metabolism, tend to be accompanied by an elevation of the basal metabolic rate. This tendency may, of course, be nullified either wholly or in part as the result of complications, such as oxygen want, which tend to depress the metabolic level.

Transfer of copper from the tissues to the blood may follow the ingestion of food rich in cuprophile substances, the hypersecretion of "copper-avid" hormones, or the disintegration of depots of strongly cuprophile bacteria. Or, it may follow the depletion of the cellular contents of glutathione, anionic ferrous iron, unsaturated fatty acids, and related substances with a high affinity for copper. Such depletion may follow the simple loss of these substances which occurs in starvation, the growth of neoplasms, and the development of fetuses, or it may follow the increased intensity of intracellular oxidation which accompanies an increase in body temperature. Substances of the glutathione, etc. type lose their high affinity for copper upon oxidation.

*Conditions in which the copper content of the serum is increased, the iron content remaining either within or below the normal range*

The copper content of the serum is increased, and the iron content decreased in conditions accompanied by a rise in body temperature. The copper content alone is changed in conditions accompanied by an elevation of the basal metabolic rate without rise in body temperature.

Sanborn (33) gives the following ranges for the basal metabolism of pregnancy, leukemia, pulmonary tuberculosis, and pernicious anemia: +33 to +35, +21 to +123, +3 to +30, and +2 to +23. Values of +10 to +50 are reported in carcinoma (34).

Hyperthyroidism and thyroid therapy were accompanied by an increase in the copper content of the serum, with no concomitant increase in iron content.

The unusually high copper level reported for the instance of pregnancy accompanied by miliary tuberculosis was observed several weeks before the existence of the complication was discovered. Comparably unusual values were twice observed in rabbits with, at the time, no other conclusive evidence of abnormality. The animals later developed large cysts and had to be sacrificed.

Normal pregnancy appeared to be associated, at term, with an in

TABLE 4

*The copper and non hemoglobinous iron contents of the serum in conditions associated with an elevation of the metabolic rate*

Description	Serum		Ratio of copper to iron
	Copper	Iron	
	micrograms per cc.	micrograms per cc.	
Normal men	80±8	100±15	0.8±2 1
Normal women	0.92±3	0.77±15	1.2±2 1
Hyperthyroidism no medication basal metabolic rate + 27 *	1.35	72	1.9 1
Hyperthyroidism receiving Lugol's basal metabolic rate + 27	1.39	28	5.0 1
Hypertension receiving oridine basal metabolic rate + 32	1.06	30	3.5 1
Pregnancy normal at term			
Maternal blood *	2.0	8	2.5 1
Fetal blood †	.53	1.6	3 1
Pregnancy at term anemia			
Maternal blood	1.4	4	3.5 1
Fetal blood	.50	7	7 1
Pregnancy at term miliary tuberculosis	3.3	.8	4.1 1
Pregnancy 7-8 months eclampsia hypertension †	2.0	1.1	1.8 1
Carcinoma			
Breast metastases anemia *	2.0	9	2.2 1
Cervix anemia slight fever	1.9	1.1	1.7 1
Rectum emaciated dying	2.3	1.0	2.3 1
Prostate bladder *	1.4	.48	2.9 1
Chorion epithelioma anemia	1.6	.23	7.0 1
Pulmonary tuberculosis ‡	1.46	.48	3.0 1
Myelogenous leukemia pulmonary edema oligoplasma	1.9	1.2	1.6 1
Myelogenous leukemia temperature + 1.5 F *	1.36	.28	4.9 1
Diabetes infection	1.9	.46	4.1 1
Diabetes head injuries temperature + 1 F	1.5	.06	25.0 1
Diabetes no medication *	1.29	.40	3.2 1
Diabetes receiving insulin *	1.06	.84	1.3 1
Lues Wassermann ++++ †	.98	.89	1.1 1
Lues cardiovascular Wassermann ++++ temperature + 1° F	1.40	.36	3.9 1



TABLE 4 (continued)

Description	Serum		Ratio of copper to iron
	Copper	Iron	
	<i>micrograms per cc</i>	<i>micrograms per cc</i>	
Normal hens ‡	14	62	2 1
Hens with Rous sarcoma ‡	19	36	5 1
Normal horses ‡	90	1 73	5 1
Horses receiving diphtheria toxin, 3-4 days after last toxin injection, temper- ature + 2 6° F	1 59	96	1 7 1
5-6 days after last toxin injection, temper- ature + 0 3° F	1 29	1 51	9 1
Horses receiving tetanus toxin, 3 days after last toxin injection, + 0 8° F *	1 28	1 08	1 2 1
5 days after last toxin injection, + 0 4° F	1 01	1 66	6 1

\* Average of 2 comparable subjects

‡ Average of 9

‡ Average of 3

§ Average of 6

|| Average of 4

crease of approximately 100 per cent in the copper content of the maternal blood serum. The copper content of the serum of blood from the umbilical cord of the fetus, at delivery, was from one-third to one-fourth that of the maternal blood, while the iron content was double.

The copper contents of the brain (35) and liver (36) of the newly born child are appreciably higher than are characteristic for the normal adult. Diffusion across the placental barrier is, apparently, sufficiently slow to permit the maintenance of a different distribution between the blood and tissues of the fetus than is maintained in the maternal body.

The copper levels observed in advanced carcinoma were analogous to those observed in pregnancy. The iron levels were not consistently reduced, in either condition, excepting in the presence of complications. Changes in the serum metal levels, analogous to those found in carcinoma in man, were found also in Barred Rock hens inoculated with Rous sarcoma number 1.

The serums of the horses were obtained through the courtesy of Eli Lilly and Co. The highest levels of serum copper, and the lowest levels of serum iron, were found in the animals reacting most severely to the toxin injections.

The highest concentrations of copper in the serums of the tuberculous subjects were present, in general, in the serums of the individuals with the

lowest expectations of recovery With one exception, the iron contents were below the normal level A similar copper iron picture was found in leukemia No uniform changes in the copper and iron levels were observed in lues in the absence of complications

The values for diabetes with and without insulin therapy, like those reported in Table 2 for pernicious anemia before and after liver therapy indicate the tendency of the organism to return to a normal copper iron balance upon the establishment of a specific therapy

#### SUMMARY AND CONCLUSION

A study has been made of the copper and non hemoglobinous iron levels in the blood serums of one hundred normal and diseased persons and of thirty two animals used for the experimental study of the factors determining the levels observed in the human subjects The analytical methods developed for the study are reported in detail are accurate, rapid and within the reach of the average clinical laboratory

The iron content of the hemoglobin free serum of normal persons from whom food had been withheld for 12 hours was found to vary from an average value of  $1.00 \pm 15$  micrograms per cc for young men to a value of  $0.77 \pm 15$  for young women Values of 1.4 microgram and above were observed in the serums of newly born infants of normal adults with mild bilirubinemia, and of persons with icterus due to cirrhosis of the liver Intermediate values were obtained in persons subjected to 24 hour fasts, and in pernicious anemia The increased values of the latter condition became corrected following the administration of liver extract Normal values were found in carcinoma, pregnancy, hypo and hyperthyroidism, and lues Values approaching 0.4 microgram and less were observed in barbitol poisoning, nephritis with edema, conditions involving pituitary and ovarian dysfunction, leukemia, pulmonary tuberculosis and diabetes The low values of the latter condition were corrected following insulin therapy Experimental recoverable depression of the iron level was obtained in rabbits subjected to hemorrhage, carbon monoxide and barbitol poisoning and in horses subjected to injections of the toxins of diphtheria and tetanus

The copper content of the blood serum of normal persons varied from an average value of  $0.80 \pm 8$  micrograms per cc for young men to a value of  $0.92 \pm 3$  for young women Values of 2.0 micrograms per cc and above were found in pregnancy, at term, and in advanced carcinoma Intermediate values were observed in toxemia pyrogenic infection, Addison's disease, leukemia, pernicious anemia cirrhosis of the liver, and diabetes Approximately normal values were obtained in lues, nephritis, and conditions associated with a depressed metabolic level excepting hypothyroidism Slightly lowered levels were observed in hypothyroidism and polycythemia vera Markedly lowered levels were manifest in the serum of newly born infants

The copper-iron ratio was below the normal averages only in conditions involving an increased serum bilirubin level. The ratio was normal in pernicious anemia, hypothyroidism, lues, and nephritis without edema. In every other condition observed, the ratio was increased, usually to a very marked degree.

An analysis of the figures reported has indicated the probable existence of four types of copper-iron picture. The first, found in incipient starvation and in pernicious anemia, may be characterized by slightly increased copper levels and markedly increased iron levels, resulting from a "burning-out" of the cell catalysts due to a depletion or deficiency in the cell nutrient of substances capable of stabilizing iron in the reduced or ferrous condition. The second, found in carbon monoxide and barbitol poisoning, and in uncomplicated anemia due to hemorrhage, may be characterized by virtually unchanged copper levels and markedly decreased iron levels, as a result of the retardation in rate of "burning-out" of the cell catalysts which accompanies a depression of the basal metabolic rate. The third, found in pyrogenic infections, toxemia, leukemia, and allied conditions associated with an elevation of the body temperature, may be characterized by increases in the copper levels and decreases in the iron levels proportionate to the general severity of the conditions. The fourth, found in pregnancy, carcinoma, and hyperthyroidism, conditions associated with an elevation of the basal metabolic rate without rise in body temperature, may be characterized by markedly increased copper levels, without accompanying change in the iron levels.

A careful examination of the serum copper-iron balance before, during, and after provisional therapy may eventually prove of definite aid in the diagnosis and treatment of disease.

The writers wish to emphasize the purely exploratory character of the work which has been presented, and the provisional nature of the classifications and hypotheses suggested. Grateful acknowledgment is made of the cooperation of Dr. Edwin F. Hirsch and others of the laboratory and staffs of St. Luke's Hospital, Eli Lilly and Co., and the Municipal Tuberculosis Sanitarium, who provided essential material and assistance.

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## CHANGES IN THE CIRCULATION PRODUCED BY GRADUAL OCCLUSION OF THE PULMONARY ARTERY

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There appears a striking lack of similarity between observations that record the effects of slow compression of the pulmonary artery in animals and the phenomena that may follow a partial occlusion of the same artery in man by a massive embolus.

Cohnheim's (1) description of the effects of slowly produced pulmonary stenosis is so classical that it is worthy of quotation particularly as other experimental studies have simply confirmed his findings. The experiments of Cohnheim recorded simultaneously the pressure in the right auricle and femoral artery. A ligature was passed about the pulmonary artery and slowly tightened. Even when the constriction was carried to a marked degree there was not "*the slightest change in the femoral curve or in the manometer in the jugular*". A change occurs only when the stenosis is carried beyond a certain point. *The arterial pressure then undergoes a steep and sudden descent*, while at the same time the level of the venous manometer rapidly rises and, if the ligature be not loosened, life is in extreme danger. This finding is used by the author to illustrate his concept of the ability of the heart to accommodate itself to increased work until a point is reached "when the resistance becomes so considerable that the cardiac contractions are no longer capable of completely overcoming it," and "the *circulation is instantly at an end*". *An intermediate state* where the blood stream, though not quite normal, continues where at each systole the heart still throws a certain quantity of blood into the arteries, though not the former normal average amount, and thus maintains the arterial and venous pressures at levels lower and higher respectively than is normally the case, such an intermediate state that is to say, as we become acquainted with in connection with increased pericardial tension *does not exist here*. The physiological heart muscle can meet the demands on its work, or it cannot meet them. In the former case, we have a regular physiological circulation, in the latter death.

The abrupt failure of the circulation under these circumstances has been described repeatedly. Haggart and Walker (2) produced occlusion of the pulmonary artery by slowly approximating the jaws of a clamp. As in Cohnheim's experiments, no effect upon systemic blood pressure or

cardiac output was observed until a certain point was reached, then the blood pressure and cardiac output fell precipitately to zero and the animal died unless the clamp was instantly released. The end point appeared so definite that these observers could predict from the number of turns of the clamp just when it was to occur. Moore and Binger (3), using a similar method, obtained identical results.

It is true that death from pulmonary embolism in man may occur with the dramatic suddenness that is registered in these experiments. The effect of complete occlusion of the pulmonary artery by an embolus can be nothing else but an immediate cessation of cardiac output and death. Not infrequently, however, instead of dying immediately, the patient lives for several hours and autopsy confirms the diagnosis of the presence of a large clot of blood partially obstructing the main stem of the artery. During the interval between embolism and death, the symptoms shown by the patient are those commonly associated with diminishing cardiac output, lowered arterial pressure, peripheral vasoconstriction, and anoxemia. In addition, increasing distention of the veins has been noted (4).

The fact that it is possible to reproduce in experiment a state of affairs comparable physiologically to that observed at the bedside was mentioned briefly in connection with a study directly concerned with the function of the pericardium (5). It is believed that the finding deserves some elaboration, however, and in the following studies we have recorded in detail the changes in arterial and venous pressures and cardiac output following partial occlusion of the main stem of the pulmonary artery. It is shown that the prolonged period of reduced cardiac output observed in man can be reproduced in the experimental animal, and that death occurs not necessarily from precipitate failure of an overloaded right heart, but from a gradual withdrawal of blood from active circulation. This blood accumulates in the venous side of the circulatory system where it is trapped by the obstruction to the outflow from the right side of the heart. Death under these circumstances occurs as it does in hemorrhage or shock—not primarily from cardiac failure, but from the effects of a reduced volume of circulating blood.

#### EXPERIMENTS

The experiments were performed on cats anesthetized by the intraperitoneal injection of a 10 per cent solution of sodium barbital, 4.5 cc per kilogram of body weight. The injection was made about forty-five minutes before the experiment was started. Drinker heart preparations (6) were used in the majority of the experiments. Cats so prepared breathe naturally with the heart exposed in the open pericardium sutured to the wall of the chest. In a few instances the pulmonary artery was exposed by removing a portion of the sternum, and artificial respiration was maintained throughout the experiment by intermittent intratracheal

insufflation. The mean carotid pressure was recorded in the usual manner with a mercury manometer. The venous tracing was obtained by the method described by Lewis and Drury (7). A solution of heparin was given intravenously to prevent clotting.

Graduated compression of the pulmonary artery was accomplished by means of a clamp which has been described in an earlier publication (5). It is very similar to the one used by Haggart and Walker, but is capable of finer adjustments.

The commonly described effect of gradual occlusion of the pulmonary artery may be reproduced with this clamp and is illustrated in Figure 1.

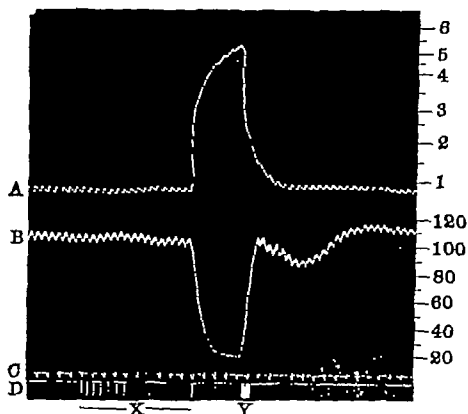


FIG 1 KYMOGRAPHIC TRACING ILLUSTRATING THE EFFECT OF COMPARATIVELY RAPID COMPRESSION OF THE PULMONARY ARTERY

A, venous pressure in centimeters of water recorded from the superior vena cava; B, arterial pressure in millimeters of mercury recorded from the left carotid artery; C, time in five second intervals; D, signal marker. The pulmonary artery was compressed during the time interval X and released at Y.

In this experiment (Number 7) the pericardium was opened and the clamp adjusted about the pulmonary artery just above the pulmonary valves. The artery was then gradually compressed by successive turns of the screw approximating the jaws of the clamp 0.635 mm at a time. Circulatory failure occurred suddenly during the course of this compression with an abrupt rise in venous pressure and a sharp fall in arterial pressure. This sudden failure of the circulation occurring in the course of gradual occlusion of the pulmonary artery is identical with that reported by other workers (1, 2, 3).

However, it was found that gradual compression of the pulmonary artery could be made to produce a slow fall in arterial pressure and a



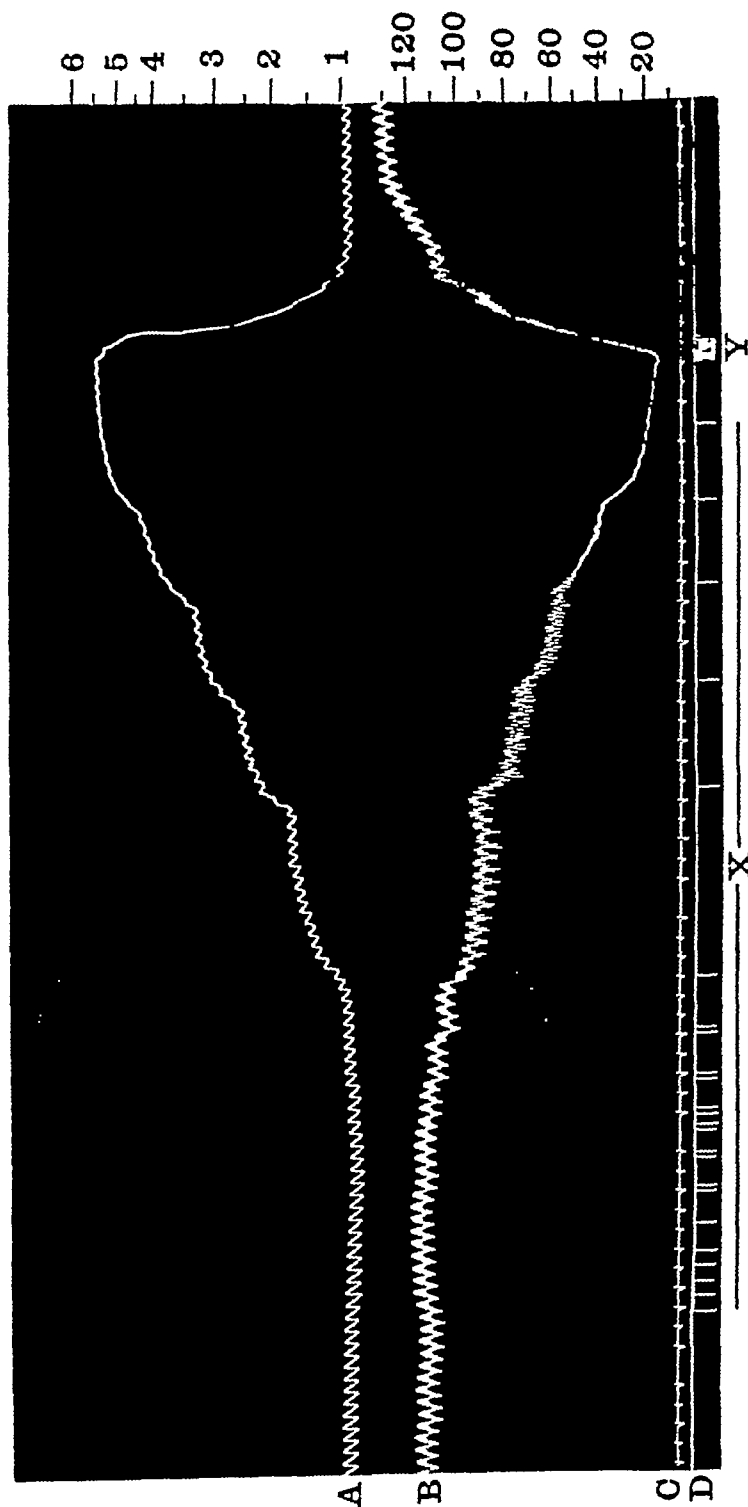


FIG 2 KYMOGRAPHIC TRACING ILLUSTRATING THE EFFECT OF MORE GRADUAL COMPRESSION OF THE PULMONARY ARTERY

A, B, C, D, as in Figure 1. During the time interval A the clamp about the pulmonary artery was tightened. The successive increments of compression were very small and a slow fall in arterial and a slow rise in venous pressure resulted. At I the clamp was completely released.



FIG 3 KYMOGRAPHIC TRACING ILLUSTRATING THE EFFECT OF GRADUAL COMPRESSION OF THE AORTA

A, B, C, D as in Figure 1. During the time interval X the clamp about the aorta was gradually tightened. The effect upon arterial and venous pressures is similar to that noted during compression of the pulmonary artery (Figure 2). The slight fall in venous and the slight rise in arterial pressure which can be observed during the initial degrees of compression were noted in many other experiments.

corresponding rise in venous pressure, provided it was carried on with sufficient *finesse*. Figure 2 is another section of the kymographic record of Experiment 7. Here the successive increments of compression were reduced to 0.079 mm and circulatory failure occurred gradually as evidenced by the slow fall in arterial pressure and rise in venous pressure. Similar results have been noted during slow and rapid compression of the aorta (Figure 3). These observations have been verified eighty-two times in forty-seven experiments in both open and closed chests and with Drinker heart preparations.

#### *Determination of cardiac output*

It seemed likely that the lowered systemic blood pressure was caused by a diminution in cardiac output resulting from forced accumulation of the blood on the venous side of the circulation. To prove this point determinations of cardiac output were made in five experiments before and during compression of the pulmonary artery. The compression was continued in each instance until the systemic blood pressure had fallen at least 10 mm Hg. The Fick method was employed to determine the minute volume flow of blood through the lungs. Drinker heart preparations were used in all five experiments. The samples of mixed venous blood were obtained by puncture of the right ventricle under direct vision. Arterial samples were taken from the femoral arteries. The tracheal cannula was connected with a closed oxygen metabolism system containing Krogh valves, a soda lime chamber and a Krogh spirometer. The fall of the spirometer gave a direct indication of oxygen consumption.

The results obtained in these five experiments are tabulated in Table I. Occlusion of the pulmonary artery sufficient to cause a drop in systemic

TABLE I  
*Cardiac output determinations with pulmonary artery occlusion*

Date	Weight of cat	Before partial occlusion of pulmonary artery		Occlusion of pulmonary artery	During partial occlusion of pulmonary artery		
		Systemic blood pressure	Cardiac output		Systemic blood pressure	Cardiac output	Decrease in cardiac output
1930	kgm	mm Hg	cc per minute	per cent	mm Hg	cc per minute	per cent
October 29	2.84	83	179		72	124	31
October 31	2.85	126	430	88	90	145	66
November 3	3.47	105	394	87	78	205	48
November 5	3.55	78	488	61	62	292	40
November 6	2.50	68	391	83	56	230	41

blood pressure of more than 10 mm Hg was accompanied by a decrease in cardiac output varying between 31 and 66 per cent. As might be expected, there was no close relationship between the degree of occlusion of the artery, the fall in systemic blood pressure and the decrease in cardiac output. However, the most marked occlusion produced the greatest fall in blood pressure and the largest decrease in cardiac output, while the smallest drop in blood pressure was associated with the smallest decrease in cardiac output.

### *Degree of occlusion produced by clamp*

In order to estimate the degree of occlusion produced by their clamp Haggart and Walker (2) compressed a section of a rubber tube and by inking the distorted end reproduced its sectional area on paper. From the measurements of these ink impressions they drew a curve from which the degree of occlusion of the pulmonary artery could be estimated. It is obvious that the accuracy obtained with such a method is not great.

The degree of occlusion of the artery was estimated with far greater accuracy in the following manner. The clamp was adjusted about the pulmonary artery at the start of each experiment so that the plane of the jaws of the clamp was at right angles to the long axis of the artery. Then the jaws were approximated until the distance between them corresponded to the diameter of the artery during systole. That is they just touched the external surface of the artery with each heart beat. At the end of the experiment, the jaws were approximated until the artery was completely occluded. The distance traversed by the lower movable jaw of the clamp from its first position to its last corresponds to the original internal diameter of the artery. The original cross sectional area of the artery may then be computed by the formula  $O = \pi R^2$  in which  $O$  represents the cross sectional area and  $R$  the original internal radius of the artery as measured by the clamp.

An elastic tube subjected to a uniform internal pressure will assume the shapes shown in Figure 4 when compressed by the clamp. By measuring a series of such figures stamped with the inked end of a rubber tube Haggart and Walker derived a curve from which they computed the degree of occlusion of the compressed artery. The formula used was  $O = \pi r^2 + 2rl$ , in which  $O$  is the cross sectional area,  $r$ , the radius of the lateral curvatures at either side of the compressed artery, and  $l$ , the transverse distance across the artery from the end of one curved edge to the beginning of the other (Figure 4 Diagram C). Now the factor  $r$  can be computed at any point in the compression because it equals one half the difference between the original diameter and the distance through which the lower jaw of the clamp has moved. But assuming the circumference of the artery to be constant,  $l$  can also be computed by the

formula  $l = \frac{2\tau R - 2\tau r}{2}$  Then substituting for  $l$  in Haggart and Walker's formula we have  $O = \pi r(2R - r)$ ,  $O$  representing the area of cross section,  $R$ , the original internal radius of the artery, and  $r$ , one-half of the difference between  $2R$  and the distance which the movable jaw of the clamp has moved toward the fixed jaw

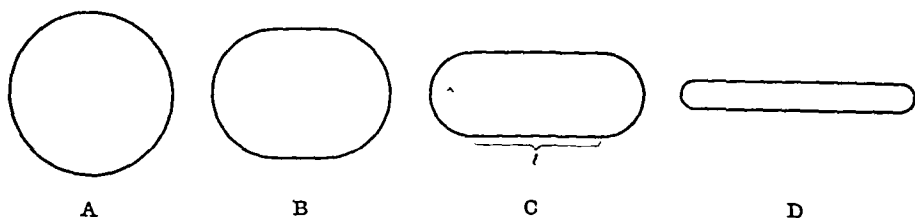


FIG 4 DIAGRAMS OF THE CROSS SECTION OF THE PULMONARY ARTERY

*A*, before compression by the clamp, *B*, *C*, *D*, successive stages of compression

The last formula makes it possible to determine directly the area of cross section of the artery with any degree of compression. The accuracy of these computed areas depends chiefly upon the care employed in placing the clamp on the artery.

In fourteen experiments determinations were made of the original cross sectional area of the pulmonary artery, the area at which the first definite lowering (10 mm Hg) of the systemic blood pressure occurred and the area at which any further compression resulted in death of the animal. It was found that the systemic blood pressure was not definitely lowered until from 61 to 86 per cent of the artery had been occluded and that the compression was not fatal until from 84 to 96 per cent of the artery had been occluded. In one instance compression was fatal when only 73 per cent of the artery had been occluded. In this experiment the animal had a large pneumothorax, which undoubtedly contributed to the circulatory failure. The variations in the figures in these fourteen experiments are probably to be accounted for by the variations in blood loss and length of the experiment with resultant differences in the ability of the right heart to overcome a partial occlusion of the pulmonary artery. The values given are all higher than the estimated degree of occlusion at which Haggart and Walker noted abrupt cardiac failure.

It is of interest to note the mechanical principles that give rise to the artifact of a finely determined end point at which circulatory failure takes place when a coarsely adjustable clamp or ligature is employed to occlude the artery. The pulmonary artery may be regarded as a flexible tube subjected to a pressure uniformly distributed upon its internal surface and the jaws of the clamp as two parallel planes compressing the tube between them. A cross section of such a tube will assume the shapes illustrated in Figure 4 as the two parallel planes approach one another.

It is evident from these diagrams that a one millimeter approximation of the jaws of the clamp when the arterial lumen resembles Diagram *D* will cause a much greater reduction in cross sectional area than a similar approximation when the lumen is of the form shown in Diagram *B*. Figure 5 is a graphic illustration of this fact. It represents the per cent

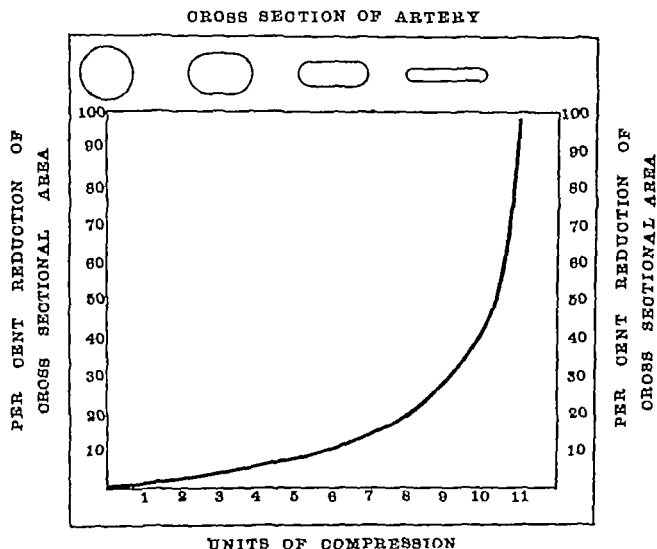


FIG 5 GRAPH OF CHANGES IN THE CROSS SECTIONAL AREA OF THE PULMONARY ARTERY DURING COMPRESSION BY THE CLAMP IN EXPERIMENT 15

Each unit of compression represents an approximation of the jaws of the clamp equal to 0.635 mm. The diagrams above the graph represent cross sections of the pulmonary artery at 0, 3, 6, and 9 units of compression.

reduction in each preceding cross sectional area of the pulmonary artery occasioned by an 0.635 mm approximation of the jaws of the clamp. Each of the first five approximations produces less than a 10 per cent diminution in the immediately preceding sectional area. Approximation by a similar increment just before complete occlusion of the artery produces a 98 per cent reduction in area. Thus, unless the clamp is capable of very fine adjustments, cardiac failure will appear to occur abruptly during a slow and gradual compression (Figure 1) although in reality it takes place when the diminution in the cross sectional area of the pulmonary artery becomes markedly increased by each turn of the clamp.

By well known hydraulic principles this rapid decrease in cross sectional area causes a sharp increase in the resistance to blood flow. A

demonstration to this effect was obtained by experiments with the excised pulmonary artery. In fourteen experiments the pulmonary artery and adjacent portion of the right ventricle were excised after the death of the cat. A glass tube having approximately the same internal diameter as the pulmonary artery was then inserted through the right ventricle and tied into the artery so that it projected just beyond the pulmonary valves. The cannula was connected with a water reservoir designed to deliver water at a constant pressure. The rate of flow through the pulmonary artery was determined by timing with a stop watch the collection of 500 cc. of water in a graduated glass cylinder. The clamp was adjusted about the pulmonary artery in much the same manner as it had been in the living cat. The rates of flow with a constant pressure in the water reservoir were determined at various degrees of occlusion of the artery.

Figure 6 is a graph of the results obtained by this method in one experiment. The same pulmonary artery was used from which the areas in Figure 5 were computed. The sharp decrease in rate of flow as com-

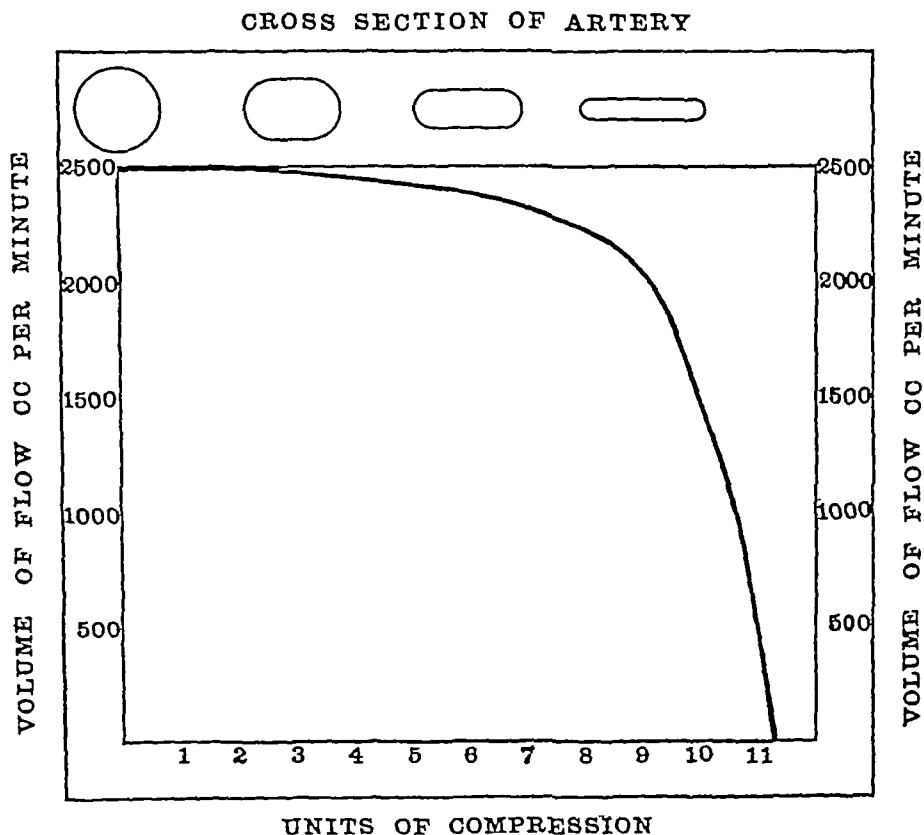


FIG 6 GRAPH OF CHANGES IN RATE OF FLOW THROUGH THE EXCISED PULMONARY ARTERY DURING COMPRESSION BY THE CLAMP

Cross sections of artery and units of compression as in Figure 5

plete occlusion of the artery was approached corresponds to the sharp diminution in the cross sectional area seen in Figure 5. In fact, by inverting Figure 6, the curves are almost superimposable. The same abrupt diminution in rate of flow was observed in the other thirteen experiments. As all other factors in these experiments were constant, the rate of flow varied inversely as the resistance offered by the clamp. This gives experimental confirmation of the sharply increasing resistance offered by the clamp already deduced from a consideration of calculated cross sectional areas. It is apparent, therefore, that the abrupt circulatory failure noted during a supposedly gradual occlusion of the pulmonary artery or aorta can be ascribed to the use of a method which in reality produces a sudden and large reduction in the calibre of the vessel after a certain point is reached.

#### CONCLUSIONS

1 Obstruction of the pulmonary artery up to 60 per cent of its cross sectional area is without significant effect upon the arterial or venous pressures.

2 A reduction in cardiac output attended by a fall in blood pressure and rise in venous pressure occurs when the occlusion lies between 60 and 85 per cent.

3 The circulation fails not primarily from cardiac insufficiency but due to the fact that blood collects on the venous side of the system by reason of the obstruction to the outflow from the right heart.

4 The obstruction is fatal when 85 to 100 per cent of the pulmonary artery is occluded.

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## STUDIES OF BLOOD GLYCOLYSIS

### I SUGAR AND PHOSPHORUS RELATIONSHIPS DURING GLYCOLYSIS IN NORMAL BLOOD<sup>1</sup>

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The series of experiments presented in this paper demonstrate certain relationships in the progressive changes of sugar and phosphorus observed in defibrinated blood incubated in vitro at 37° C through periods of from ten to twenty four hours. The accompanying figures illustrate a pattern of these changes which may be regarded as characteristic of normal bloods and bloods of normal animals rendered hyperglycemic and hypoglycemic. Similar studies of glycolysis and phosphorus relationships observed in blood samples from children with a variety of diseases will be presented in the succeeding paper.

Most of the evidence that phosphorus plays an essential rôle in the intermediary carbohydrate metabolism of the animal body is based upon observations of shifts of phosphorus in the body and fluctuations in the excretion of phosphorus which accompany the storage and burning of sugar. This subject has been recently reviewed by Peters and Van Slyke (1931). It is generally believed that there is formation of carbohydrate phosphoric acid compounds at certain stages in the processes of storage (as in the synthesis of glycogen) and burning of sugar, but the actual isolation of such compounds from animal tissues has proved difficult and the evidence that such compounds exist in the animal body is for the most part indirect. Goodwin and Robison (1924) reported the isolation of two phosphoric esters from the blood; one of these esters reduced Fehling's solution. The existence of hexose-phosphoric acid compounds in yeasts was demonstrated by Harden and his collaborators (1905-1923), Robison (1922), Levene and Raymond (1928, 1929) and the rôle of phosphorus in alcoholic fermentation of sugar by yeasts was clearly defined by Harden's studies (1923). The manner in which phosphorus enters into the splitting of sugar by yeast enzymes is better understood than are the processes by which sugar is utilized in the animal body but certain steps of the chemical mechanisms in the respective organisms are probably closely analogous and much that has been learned from the one has been profitably applied by a number of investigators in studying the other.

The earliest suggestion that phosphorus might be concerned in blood glycolysis appears to have been made by Rona and Döblin (1911), who seized

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<sup>1</sup> An abstract of this paper was read at a meeting of the American Pediatric Society, Montreal June 18, 1930.

upon the observations of Löb (1911) that sodium phosphate when added to an alkaline solution of glucose hastened the spontaneous destruction of the glucose. Interrelationships between phosphorus and sugar in the blood during glycolysis have been demonstrated in a number of investigations reported by Rona and his collaborators (1911-1927), Bierry and Moquet (1924, 1925), Lawaczek (1924), Martland (1925), Martland, Hansman and Robison (1924), Jost (1927), Engelhardt and Braunstein (1928), Barrenscheen and Hubner (1930), Engelhardt and Ljubimowa (1930) and Roche and Roche (1927, 1929). Briefly summarized, these investigations have demonstrated the following points:

When freshly drawn blood is defibrinated and incubated, the blood sugar progressively diminishes. During the first few hours the inorganic phosphorus may diminish slightly or may remain at a stationary level. When glycolysis slows, or stops, from lack of sugar, there is a rapid liberation of inorganic phosphorus from the organic phosphorus compounds of the cells. There is general concurrence in the explanation, as stated by Engelhardt and Braunstein (1928) and by Roche and Roche (1929), that during blood glycolysis two main reactions are going on—namely, synthesis of hexose-phosphoric acid esters as a first step in glycolysis, and the hydrolysis of these esters. These reactions are interrelated, but the hydrolysis may proceed independent of synthesis. According to the speed of the two reactions there is observed 1, excess of synthesis over hydrolysis, with fixation of the inorganic phosphorus in organic form, 2, equality of the two reactions, 3, excess of hydrolysis over synthesis, resulting in liberation of inorganic phosphorus.

The processes of glycolysis, ester-synthesis and ester-hydrolysis are affected differently by various changes in the state of the blood. Most of the glycolysis is due to the erythrocytes and is probably intracellular, hence, apart from the chemical state of the blood, the number of cells in a given sample is an important factor in glycolysis (Katayama (1926), Kawashima (1923), Barer (1931)). No glycolysis takes place in the plasma or serum if precautions are taken to avoid damage to the cells and the escape of their contents into the serum during its preparation (Rona and Döblin (1911), Milne and Peters (1912), Kawashima (1923)). Hemolysis of the blood with water stops glycolysis (Doyon and Morel (1903), Rona and Döblin (1911)) and ester synthesis, but does not stop phosphoric-ester hydrolysis (Martland, Hansman and Robison (1924), Lawaczek (1924)). Dilution of blood with isotonic salt solution merely slows glycolysis. Dilution of the cells with serum or a phosphate mixture gives greater glycolysis than equal dilution of the cells with NaCl or Ringer's solution (Kawashima (1924)). The addition of glucose and phosphate in an appropriately buffered mixture to the whole blood gives greater synthesis of esters and increased speed of glycolysis (Roche and Roche (1929)). The optimum reaction for glycolysis is around pH 7.8—approximately the optimum for ester synthesis. According to Martland (1925) and Rona and Iwasaki (1927), ester synthesis and glycolysis take place only at an alkaline reaction, and are slowed by a shift to below pH 7.3. Ester hydrolysis occurs over a greater range than glycolysis, from pH 6.0 to 9.0, but in the absence of sugar, hydrolysis is most rapid at around pH 8.0 (Rona and Iwasaki (1927)).

Discussion of the nature and distribution of the various phosphorus compounds in blood may be found in the papers of Kay and Byrom (1927) and in the recent review by Peters and Van Slyke (1931). Studies of the phosphorus distribution in the blood of dogs have been reported in this journal by Guest and Andrus (1932). In normal blood the inorganic P has a slightly lower concentration in the cells than in the plasma, while the organic acid-

soluble P (the fraction designated "Ester-P" by Kay and Byrom) is practically confined to the cells there being usually less than 0.5 mgm per cent present in the plasma.

#### METHODS

Blood samples were defibrinated with a glass rod or a wooden stick and incubated at 37° C in an Erlenmeyer flask with a rubber stopper. At the intervals indicated, 1.0 or 2.0 cc samples were removed for sugar and phosphorus determinations, and the changing values thus determined are shown graphically in the figures.

Sugar determinations were made using the Folin Wu (1919) copper solution and the molybdate solution modified by Folin (1926). Phosphorus determinations were made by the Fiske Subbarow method (1925).

Precautions to maintain sterility were not taken after suitable controls had indicated that slight bacterial contamination did not alter the cycle of chemical changes dealt with here. Mackenzie (1915), Katayama (1926) and Falcon-Lesses (1927) found that slight bacterial contamination did not affect the glycolytic rate.

#### RESULTS

##### *Glycolysis and changes of phosphorus in normal blood (Figures 1 and 2)*

Glycolysis usually occurs in normal blood at a uniform rate of from 13 to 16 mgm per cent loss of sugar per hour, continuing until there remains a residual reducing substance equivalent to about 20 mgm per cent of sugar. See Figure 1. Sometimes the rate is slightly faster during the first hour, and it may slow considerably during the last hour or two. The residual substance apparently corresponds to the unidentified reducing substance of the blood which is not fermented by yeast (Hiller, Linder and Van Slyke (1925), Folin and Svedberg (1926)). When determined by different methods this substance gives considerably different values, Falcon-Lesses (1927) using Folin's (1926) copper solution for sugar determination found 6 to 8 mgm per cent of non glycolyzing reducing substance in blood. Whatever method of sugar determination is used, however, the relative changes which determine the rate of glycolysis are the same. During the first few hours the inorganic phosphorus either remains at a constant level or diminishes slightly. At the end of about 6 hours, when the sugar is exhausted the inorganic phosphorus rises sharply and progressively to reach finally a concentration of 20 to 25 mgm per cent at about the 20th hour, after which time it changes very little.

The source of the inorganic phosphorus which is found increasing rapidly after the 6th hour of incubation (as in Fig. 1) is demonstrated by the more complete analyses of the succeeding experiment. In the experiment shown in Figure 2, 150 cc. of blood from a normal man were defi-

brinated and incubated as usual. Values for the sugar content and the distribution of phosphorus in the blood as the inorganic P, total acid-soluble P and total P, were determined at the intervals indicated in Figure 2 over a period of 22 hours. Glycolysis in this blood averaged 13.5 mgm per cent loss of sugar per hour and was complete at about the 6th hour. The inorganic P diminished slightly during the first 3 hours and rose sharply after the 6th hour, this rise starting just before glycolysis was completed. The total P and the acid soluble P were unchanged during the 22 hours, it appears therefore that the acid-insoluble P (the difference between these two values) is not affected during this period of incubation.

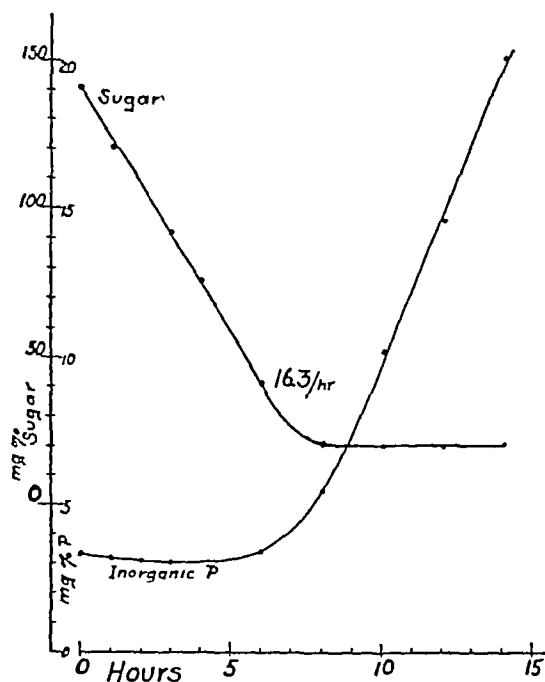


FIG 1 GLYCOLYSIS AND CHANGES OF INORGANIC PHOSPHORUS IN NORMAL HUMAN BLOOD, DEFIBRINATED AND INCUBATED AT 37° C

The values for the organic acid-soluble P ("ester-phosphorus"), represented in the figure by the dotted curve, were obtained by subtracting the inorganic P values from those of the total acid-soluble P, and it may be seen from these changing values that the increase of the inorganic P after the 6th hour is at the expense of the organic acid-soluble "ester-phosphorus". Parallel measurements made on the serum showed that the inorganic P escaped from the cells as rapidly as it was liberated from the organic compounds, but for the sake of brevity this phase of the problem is omitted from the present discussion.

According to theories advanced by the authors cited in the introduction the order of events visualized in these two figures is probably as

follows Through enzyme synthesis, free sugar is combined with free inorganic phosphate to form hexose phosphoric acid esters in the cells As the glycolytic enzyme (or enzymes) splits the sugar molecule (first to

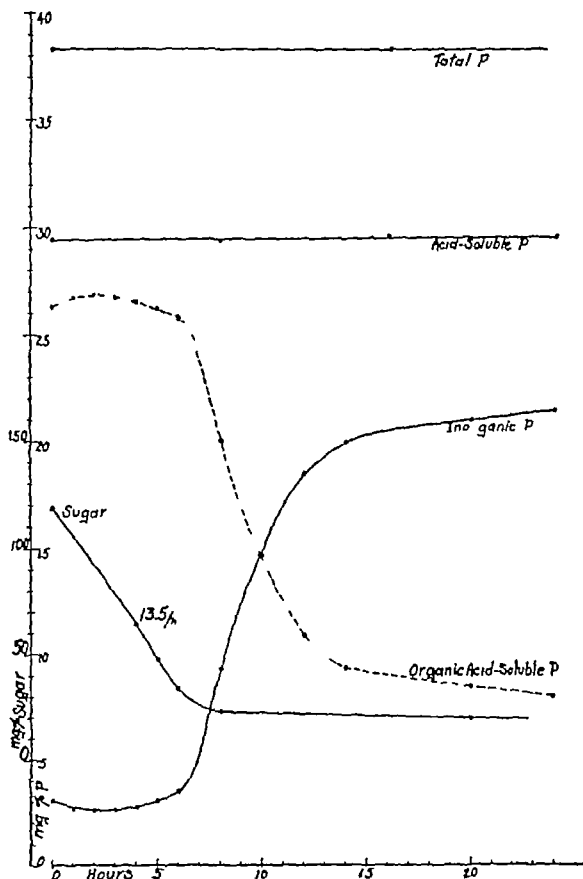


FIG 2 CHANGES IN PHOSPHORUS PARTITION DURING GLYCOLYSIS IN NORMAL HUMAN BLOOD, DEFIBRINATED AND INCUBATED AT 37° C

two molecules of lactic acid) the phosphate is liberated to combine again with sugar. Synthesis and hydrolysis in the blood are at first about equal, and while sugar is present the concentration of inorganic P changes

very little. When the sugar is exhausted, the inorganic P increases rapidly because of the continuous hydrolysis of the esters with no sugar available for their resynthesis.

That the concentration of sugar in the blood has an important bearing upon the time relationships of the changes thus observed is demonstrated in the following experiments in which the sugar content of different bloods was changed by the addition of sugar to the blood *in vitro*, and by the administration of insulin to diminish the sugar content of the blood *in vivo*.

*The effect of changes in the sugar content of normal blood upon the order of changes of inorganic phosphorus during glycolysis (Figures 3 to 8, inclusive)*

Levulose was used in the experiments that follow because this sugar gave somewhat more clear cut results than did glucose. There are, however, only slight differences between the behavior of glucose and levulose in such experiments as these.

In the experiment shown in Figure 3, 100 cc of blood from a normal man were defibrinated and incubated as usual. Sugar and inorganic P determinations were made at the intervals indicated by the graphs ( $S_1$  and  $P_1$ ). At the 5th hour the blood was divided, and 30 cc transferred to each of two flasks, to one was added 1.0 cc of 6.0 per cent (approximate) levulose in 0.85 per cent NaCl solution, sufficient to elevate the total sugar content in this blood from 36 mgm per cent to 234 mgm per cent as shown ( $S_2$ ), the other flask received an equal amount of 0.85 per cent NaCl solution. Glycolysis of the added levulose ( $S_2$ ) went on at practically the same rate as that at which the original blood sugar ( $S_1$ ) had disappeared, and the inorganic phosphorus ( $P_2$ ) in this blood continued to diminish slowly up to the 11th hour when the experiment was discontinued. In the blood sample to which no sugar was added, the inorganic phosphorus ( $P_1$ ) rose sharply as soon as glycolysis was completed.

The above experiment was repeated, using a larger sample of blood in order to continue the measurements over a longer period of time. The blood was defibrinated and incubated as usual and determinations of the sugar and inorganic P content were made at the intervals indicated in Figure 4. At the 5th and 15th hours, 20 cc of blood were removed from the original flask to smaller flasks and levulose added to these flasks in sufficient quantity to raise the sugar content of the blood in each to the levels indicated by  $S_2$  and  $S_3$ . Glycolysis of the added sugar proceeded at nearly equal rates in each case. In the untreated blood the inorganic P rose after the 6th hour, at which time the glycolysis was becoming considerably slower ( $S_1$ ). In the blood with added sugar ( $S_2$ ) the inorganic phosphorus ( $P_2$ ) remained low and was rising only slowly.

from the 18th to the 20th hour. At the 15th hour the inorganic phosphorus ( $P_i$ ) in the untreated blood was above 17.5 mgm per cent and increased to 19.7 mgm per cent at the 22nd hour. Following the addition of levulose to the blood at the 15th hour, glycolysis proceeded at a regular rate ( $S_2$ ) and the inorganic phosphorus ( $P_2$ ) in this blood diminished to 15.5 mgm per cent at the 22nd hour when the experiment was discontinued.

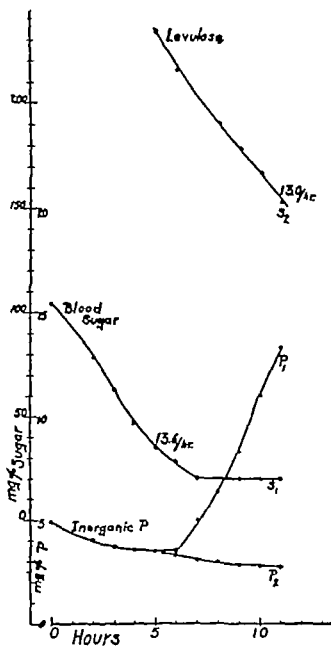


FIG 3 LEVULOSE ADDED TO DEFIBRINATED HUMAN BLOOD AT THE 5TH HOUR OF INCUBATION

From these two experiments one may conclude that in normal blood an excess of sugar, added in order to prolong the period of glycolysis, delays the rise of the inorganic phosphorus. The fact that the addition of sugar to the blood at the 15th hour caused the inorganic phosphorus to diminish, instead of further increasing, indicates that the enzymes were active and capable of ester synthesis long after the glycolysis of the original blood sugar was finished, and after ester hydrolysis had increased the inorganic phosphorus to a high level.



The curves  $S_1$  and  $P_1$  in Figure 5 illustrate the time relationships between changes in sugar and inorganic phosphorus which were observed during glycolysis in the blood of a normal rabbit. Another rabbit was given 20 units of insulin to provoke a hypoglycemia, and a blood sample was taken when the rabbit was having mild convulsions. The curves

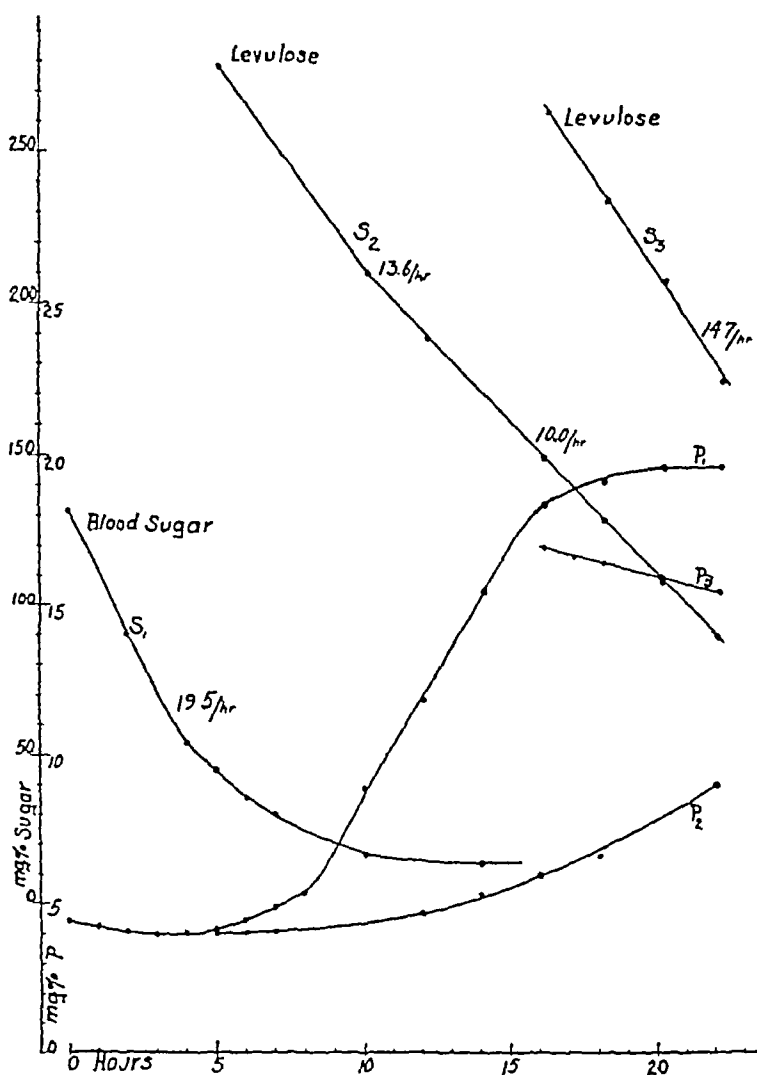


FIG 4 LEVULOSE ADDED TO DEFIBRINATED HUMAN BLOOD AT THE 5TH AND 15TH HOURS OF INCUBATION

$S_2$  and  $P_2$  represent glycolysis and ester-phosphorus hydrolysis observed in this blood. The initial effect of insulin in animals is a depression of the inorganic phosphorus of the blood (Wigglesworth, Woodrow, Smith and Winter (1923), Harrop and Benedict (1924)), but Briggs, Koechig, Doisy and Weber (1923) observed that in the stage of marked hypoglycemia and irritability (convulsions) the inorganic phosphorus tended to

rise again above its original level. The changes illustrated by the curve  $P_2$  in the figure offer an explanation for the findings of these authors. Even *in vivo*, if the blood sugar is thus reduced by insulin, ester phosphorus hydrolysis probably leads to an increase of the inorganic phosphorus of the blood when there is a lack of sugar available for resynthesis of the esters.

In order to show definitely that the rapid ester phosphorus hydrolysis observed in this hypoglycemic blood was in fact due to the lack of sugar

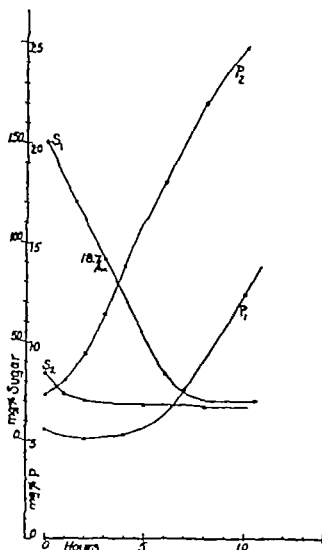


FIG 5 GLYCOLYSIS AND CHANGES OF INORGANIC PHOSPHORUS IN DEFIBRINATED NORMAL RABBIT'S BLOOD ( $S_1$  AND  $P_1$ ), AND IN THE BLOOD OF A RABBIT RENDERED HYPOLYCEMIC BY INSULIN ( $S_2$  AND  $P_2$ )

rather than to some effect attendant upon the insulin injection, sugar was added *in vitro* to another sample of rabbit's blood that had been rendered hypoglycemic in the same fashion, the result of this experiment is shown in Figure 6. Twenty units of insulin given in divided doses to a rabbit reduced the blood sugar content from 147 mgm per cent to 24 mgm per cent. The animal was having mild convulsions when a sample of blood was drawn from the heart. The blood sample (40 cc.) was defibrinated and divided into two flasks, to one flask was added 0.5 cc. of 10 per cent (approximate) levulose solution. The flasks were incubated as usual

and sugar and inorganic phosphorus determinations were made at the intervals indicated in Figure 6. In the blood in which the sugar content was only 24 mgm per cent, glycolysis was rapidly completed ( $S_1$ ) and the inorganic P ( $P_1$ ) rose from the start of incubation, in the blood to which levulose was added glycolysis occurred at a regular rate ( $S_2$ ) and the inorganic phosphorus ( $P_2$ ), after a slight initial rise, remained low and was increasing only slowly after the 11th hour.

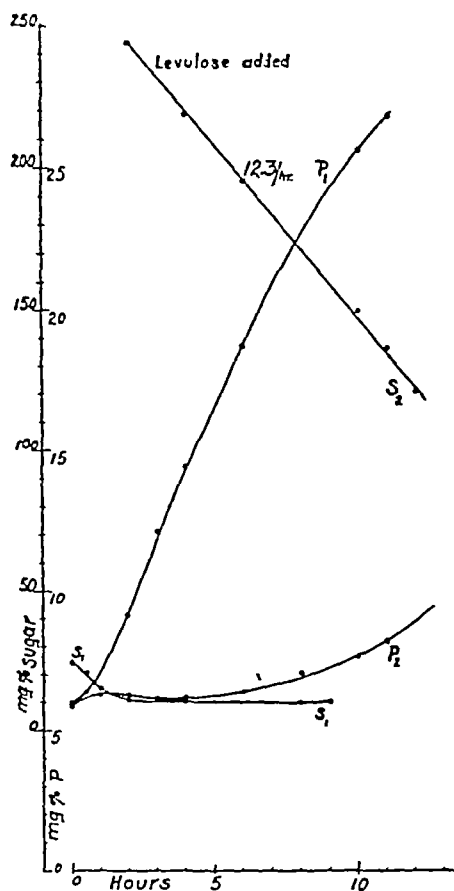


FIG 6 CHANGES OF SUGAR AND INORGANIC PHOSPHORUS IN THE BLOOD OF A RABBIT RENDERED HYPOGLYCEMIC BY INSULIN ( $S_1$  AND  $P_1$ ), AND IN THE SAME BLOOD WITH LEVULOSE ADDED ( $S_2$  AND  $P_2$ )

In Figures 7 and 8 are shown curves representing glycolysis and changes of inorganic phosphorus in blood samples taken from two diabetic patients, one with high and the other with low blood sugar content. Both patients had been receiving insulin and were under good dietary control and appeared to be in a healthy state when these blood samples were taken. In the first patient mild hyperglycemia was allowed to develop by withholding insulin, there was marked glycosuria at the time the

blood sample was taken, but no acetone appeared in the urine. The blood sample taken at this time, with sugar content 292 mgm per cent, was defibrinated and incubated as usual, determinations of the sugar and inorganic phosphorus made at intervals during a period of 18 hours gave the results shown in Figure 7. From the second patient a blood sample was taken after a dose of insulin had been given to induce a mild hypo-

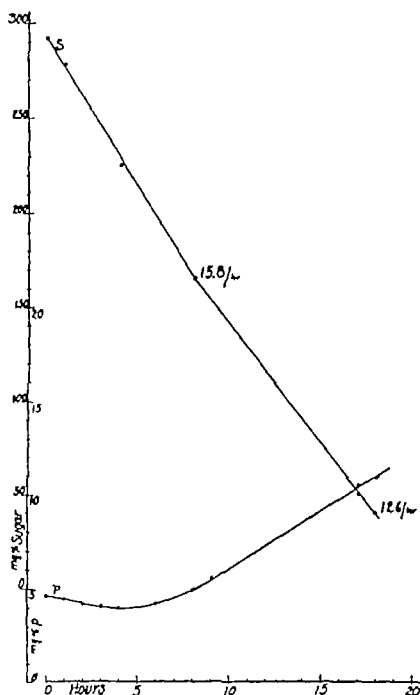


FIG 7 GLYCOLYSIS AND CHANGES OF INORGANIC PHOSPHORUS IN THE HYPERGLYCEMIC BLOOD OF A DIABETIC MAN

glycemia. This blood sample, with sugar content 65 mgm per cent, was defibrinated and divided into two flasks (10 cc. in each), to one of which was added 0.2 cc. of 10 per cent levulose, sufficient to elevate the blood sugar to 260 mgm per cent as shown by  $S_2$  in Figure 8. Glycolysis occurred at practically the same rate in both samples ( $S_1$  and  $S_2$ ). With the completion of glycolysis in the sample to which no sugar was added, the inorganic phosphorus ( $P_1$ ) rose sharply after the second hour, while

in the blood to which levulose was added the inorganic phosphorus ( $P_2$ ) was progressively diminishing through the 6th hour when the experiment was discontinued

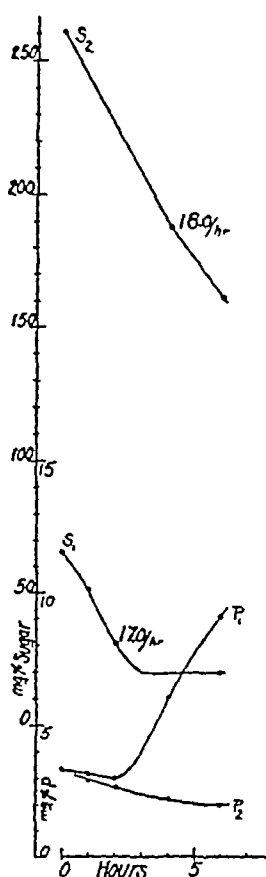


FIG 8 BLOOD OF A DIABETIC CHILD RENDERED HYPOGLYCEMIC BY INSULIN ALONE ( $S_1$  AND  $P_1$ ) AND WITH LEVULOSE ADDED ( $S_2$  AND  $P_2$ )

*The effect of added phosphate upon blood glycolysis (Figure 9)*

In the foregoing experiments are demonstrated the effects of a changed sugar concentration upon the changes of inorganic P in normal blood. The effect of an increased inorganic phosphate concentration upon glycolysis is displayed in Figure 9. Fifty cc of blood were taken from a normal adult, defibrinated, and divided into two flasks. To one was added 2.0 cc of 0.85 per cent NaCl solution. To the other was added 2.0 cc of 1.2 per cent (approximate) solution of  $Na_2HPO_4 \cdot 12H_2O$ , sufficient to elevate the inorganic phosphorus content of the blood from 3.1 to 17.8 mgm per cent. In the blood sample with the added phosphate, glycolysis occurred at a slightly faster rate ( $S_2$ ) than it did in the control ( $S_1$ ), and in this sample the inorganic phosphorus ( $P_2$ ) was decreased more during the first 4 hours than in the control ( $P_1$ ).

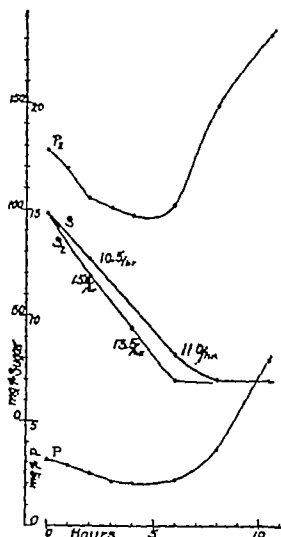


FIG 9 GLYCOLYSIS AND CHANGES OF INORGANIC PHOSPHORUS IN NORMAL HUMAN BLOOD ( $S_1$  AND  $P_1$ ) AND IN THE SAME BLOOD WITH  $Na_2HPO_4$  ADDED ( $S_2$  AND  $P_2$ )

### SUMMARY

In normal defibrinated blood incubated at  $37^\circ C$ , glycolysis occurs at a fairly constant rate, usually amounting to a loss of 13 to 16 mgm per cent per hour until there remains a residual reducing substance of about 20 mgm per cent. During the first few hours the inorganic phosphorus of the blood either remains at a constant level or diminishes slightly. At the time the free sugar is exhausted (after 6 to 8 hours) the inorganic phosphorus rises sharply and progressively to reach a concentration of about 20 to 25 mgm per cent at about the 15th hour. This rise of the inorganic phosphorus is at the expense of the organic acid-soluble phosphorus of the cells, generally designated "ester phosphorus". The rise occurs very quickly in the hypoglycemic blood of insulinized animals, it is delayed for several hours by the addition of an excess of levulose or dextrose to both normal and hypoglycemic blood.

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## STUDIES OF BLOOD GLYCOLYSIS

### II SUGAR AND PHOSPHORUS RELATIONSHIPS DURING GLYCOLYSIS IN THE BLOOD OF INFANTS AND CHILDREN WITH VARIOUS DISEASES<sup>1</sup>

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The significance of the rate of blood glycolysis as it occurs in health and in disease has been the subject of a great many investigations which are cited in the articles by John (1925), Falcon Lesses (1927) and Barer (1931). The rate of blood glycolysis is fairly constant in health but varies greatly in certain conditions of disease, the most rapid rates having been observed in leukemia (Falcon Lesses (1927)). Acceleration of the glycolytic rate has been observed also in diabetes, in heart disease, in nephritis, in septicemia, and in other unrelated conditions. However, it is likely that the acceleration of blood glycolysis observed in these conditions is not a peculiarly characteristic manifestation of any of the diseases named, but is due to some common changes in the state of the blood. In previously reported clinical studies, comparatively little attempt has been made to correlate with the determinations of blood glycolysis other chemical alterations of the blood which might influence glycolysis, and apparently no attention has been paid to the behavior of the blood phosphorus during glycolysis in different pathologic conditions.

In many of the diseases in which alterations of the rate of glycolysis have been reported, marked changes of the blood phosphorus are known to occur and some abnormality of the phosphorus metabolism often may be recognized. The studies to be reported here were undertaken with the idea that in those pathologic conditions in which glycolysis is altered the related order of changes of the blood phosphorus observed in normal blood during glycolysis (as described in the preceding paper) might also be found altered.

#### METHODS

The methods employed were the same as those mentioned in the preceding paper.

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<sup>1</sup> An abstract of this paper was read at a meeting of the American Pediatric Society, Montreal, June 18, 1930.

## RESULTS

The glycolysis and inorganic P curves shown in the first eight of the figures may be regarded as nearly normal, they are presented to demonstrate the slight variations in the pattern of these changes which may be observed in the milder disturbances of various diseases. More marked alterations of the normal pattern of the glycolysis and inorganic P curves are shown in Figures 9 to 17, inclusive.

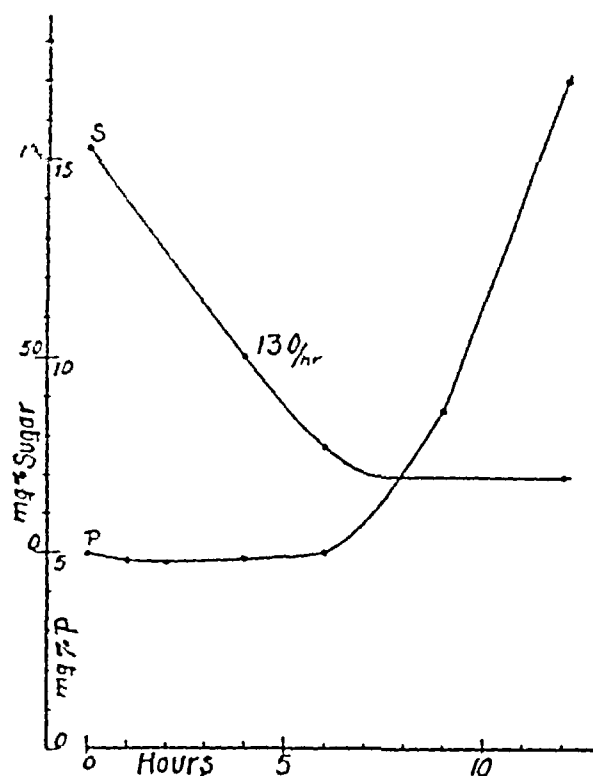


FIG 1 PURPURA HEMORRHAGICA

E. D., female, 6½ years, not acutely ill

*Case 1, Figure 1* Thrombocytopenic hemorrhagic purpura E. D., female, age 6½ years, not acutely ill. Physical findings were normal except for extensive purpuric lesions over the body. The bleeding time was markedly prolonged and the clotting time of the blood was normal. The curves representing changes of sugar and inorganic P during glycolysis in this blood may be accepted as characteristic of normal blood. Note that there was little change in the inorganic P until after the sugar was exhausted.

*Case 2, Figure 2* Celiac disease P. T., male, age 4 years, not acutely ill. Typical history of celiac disease, with onset at about one year of age. He had been under treatment several months and was doing well when this blood sample was taken.

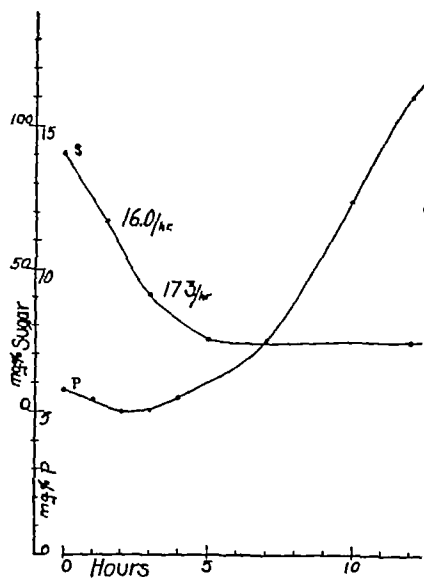


FIG 2 CELIAC DISEASE

P T, male, 4 years, not acutely ill

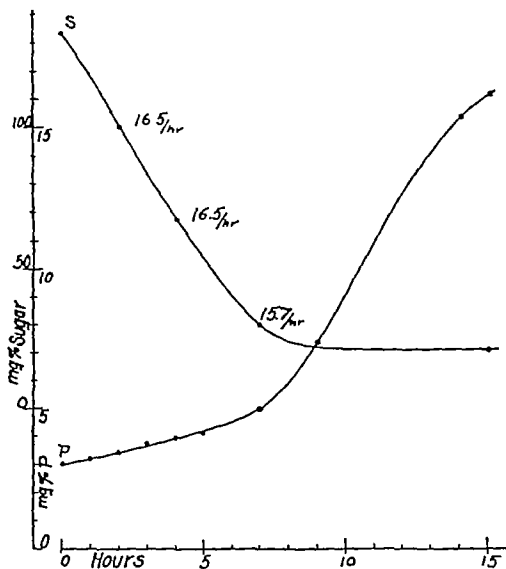


FIG 3 RICKETS

A J, female, 3 years, not acutely ill

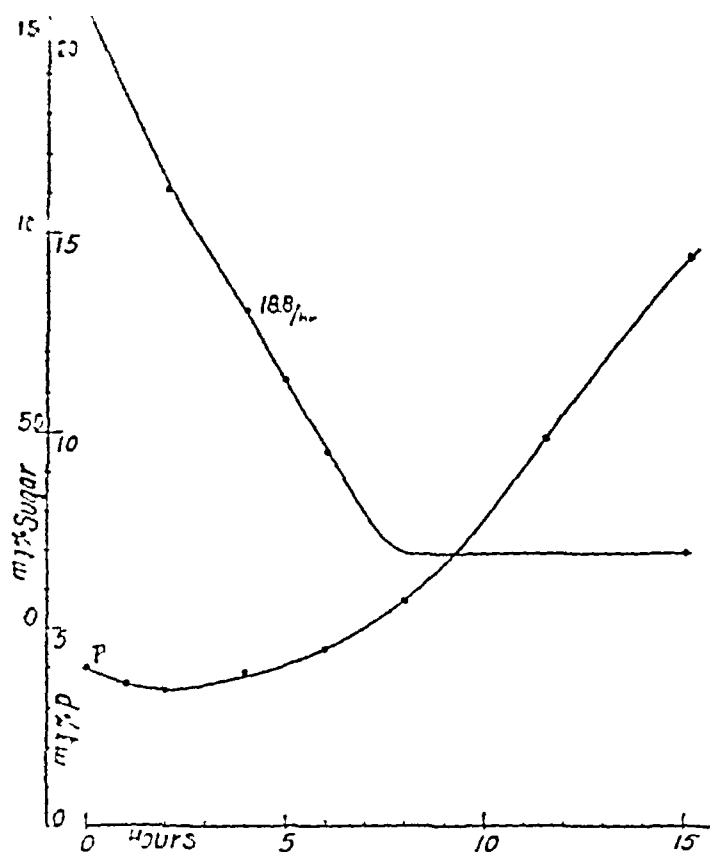


FIG 4 TUBERCULOUS MENINGITIS

J F B, male, 1 year

5 hours prior to admission, and were practically continuous until death occurred 4 days later. The blood sample was taken 2 days before death, when the temperature was  $103^{\circ}\text{F}$ . Glycolysis and inorganic P curves were essentially normal. Normal curves were likewise obtained from blood samples of 3 other infants with the same diagnosis and similar symptoms.

Case 5, Figure 5. Upper respiratory infection, with recovery. J McC, male, age 3 years, well developed and nourished, complaining of sore throat. Temperature was  $103^{\circ}\text{F}$ . Physical examination was essentially negative except for redness of pharynx. Lungs were clear. Temperature fell to normal after 3 days. In Figure 5 the curves  $S_2$  and  $P_2$  are from a blood sample taken the

day after admission to the hospital,  $S_2$  and  $P_2$  are from a sample taken 5 days later, after apparent complete recovery

*Case 6, Figure 6 Dysentery (Flexner type) F M, male, age 6 years, acutely ill* Temperature was  $104^\circ$  F Preceding admission to the hospital there had been diarrhea, fever, and headache of 2 days duration Stool culture yielded Flexner dysentery bacillus, and 3 weeks later the blood serum agglutinated a standard antigen of this organism The curves in Figure 6 are from a blood sample taken on the day of admission The inorganic P rose slightly during the first 3 hours before glycolysis was completed, but the pattern of the curves is nearly normal

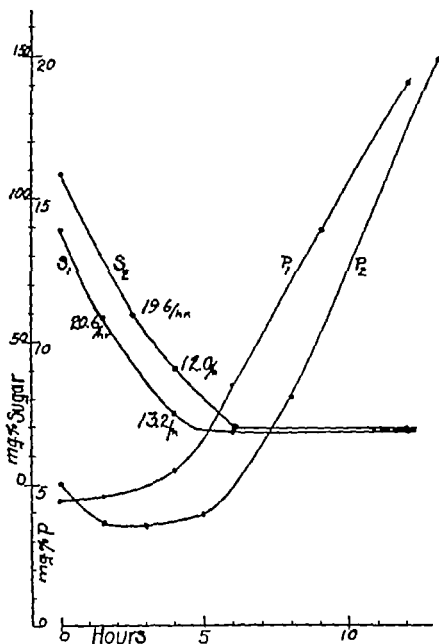


FIG 5 UPPER RESPIRATORY INFECTION

J McC, male, 3 years  $S_1$  and  $P_1$ , from blood taken during the acute infection  $S_2$  and  $P_2$ , from blood taken 5 days later, after recovery

*Case 7 Figure 7 Typhoid fever W S, male, age 3 years* Acute onset of symptoms was 2 weeks previously Stool culture yielded *B typhosus*, and the Widal test became positive There was a typical course of typhoid fever, moderately severe, with slight improvement at the time the blood sample was taken for the glycolysis studies Temperature at this time was  $101^\circ$  F, and fell to normal about 10 days later Glycolysis and inorganic P curves were

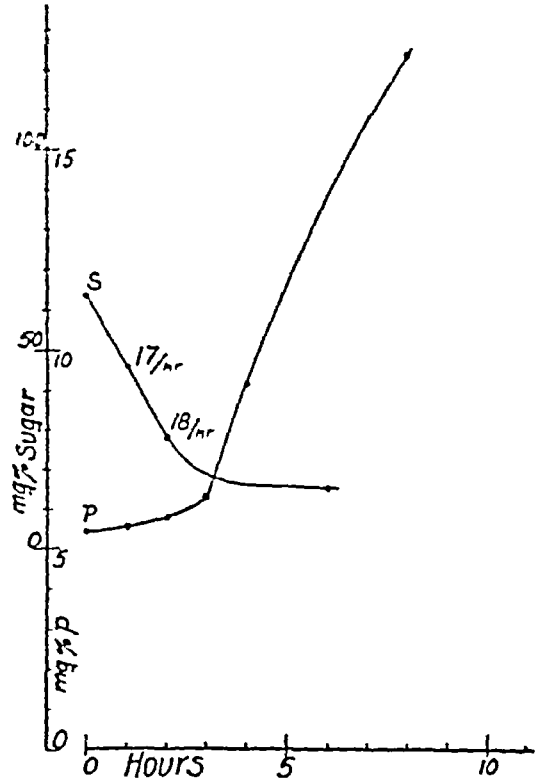


FIG 6 DYSENTERY FLENNER TYPE

F M , male, 6 years, acutely ill

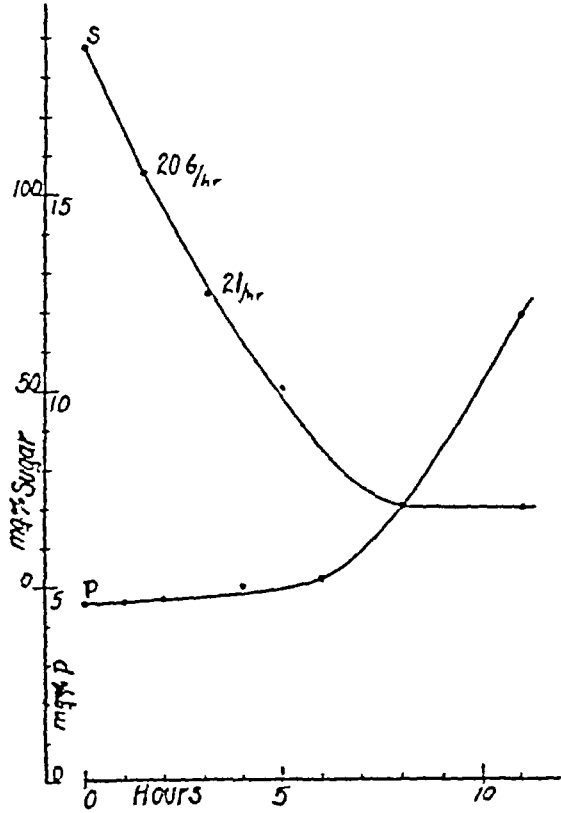


FIG 7 TYPHOID FEVER

W S , male, 3 years, blood sample taken at about the middle of the period of the illness

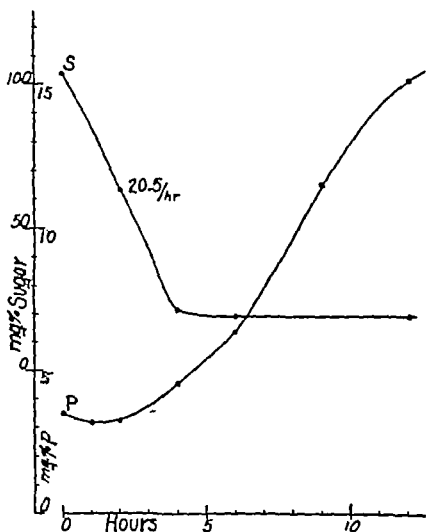


FIG 8 LEAD POISONING

K T, male, 18 months blood sample taken 48 hours antemortem

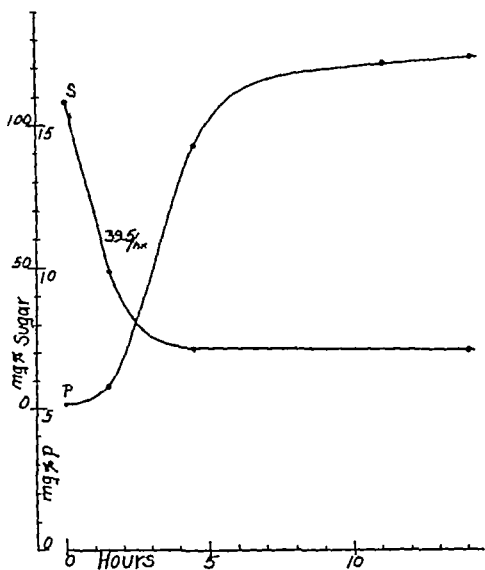


FIG 9 LEAD POISONING

R S, male, 18 months, blood sample taken 20 hours antemortem



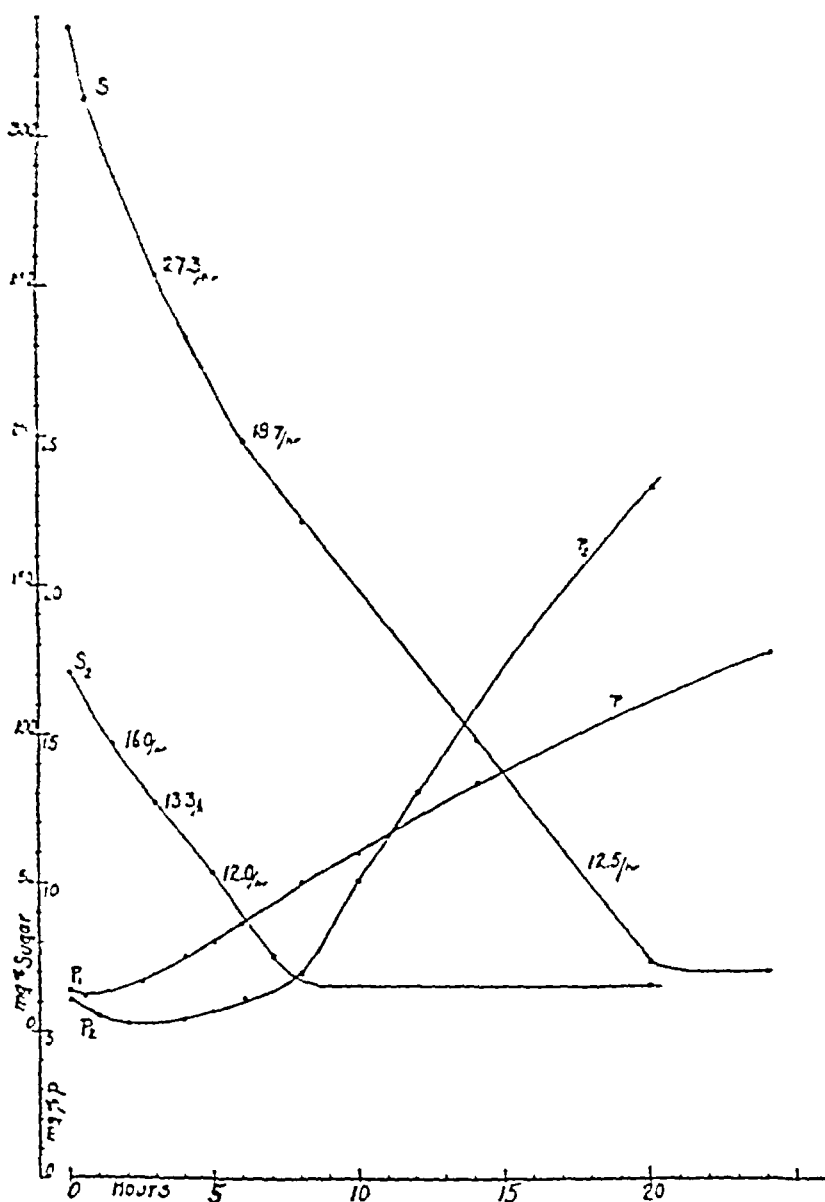


FIG 10 ENCEPHALITIS WITH HYPERGLYCEMIA

H H, male, 7 years,  $S_1$  and  $P_1$ , from a blood sample taken at time of admission to hospital,  $S_2$  and  $P_2$ , from a blood sample taken a week later

essentially normal, although the rate of glycolysis was slightly accelerated (21 mgm per cent loss per hour)

Case 8, Figure 8 Lead poisoning K T, male, age 18 months History of eating paint from furniture Patient had been vomiting once a day for 2 weeks and became stuporous 24 hours before admission to the hospital There were no convulsions (until after this blood sample was taken) Patient had fever and mild pharyngitis Stained blood film showed many stippled cells Patient died 2 days after admission

*Case 9, Figure 9 Lead poisoning R S, male, age 18 months* History of eating paint. Onset of symptoms with occasional vomiting was 2 months previously. Two hours before admission the infant vomited, became unconscious and was having continuous mild convulsions when brought to the hospital. Stippled cells 4.2 per cent. Patient died 24 hours after the blood sample was taken for glycolysis studies. Glycolysis was rapid, 39.5 mgm per cent loss per hour.

*Case 10, Figure 10 Hyperglycemia of encephalitis, with recovery H H,*

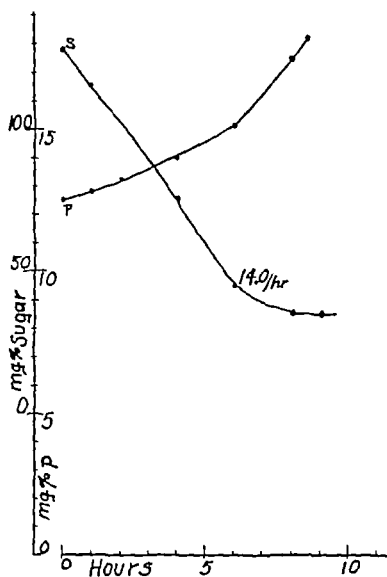


FIG 11 ACUTE NEPHRITIS

J K., male, 10 years, on verge of coma blood sample taken about 12 hours antemortem

male negro, age 7 years, admitted to the hospital in an unconscious state with a history of sudden onset of convulsions beginning 2 hours previously. Temperature was  $102^{\circ}\text{F}$ . Spinal fluid contained fresh blood, but was sterile on culture. On the second day he was quieter, but did not regain complete consciousness until the 8th day. Right-sided weakness persisted for about 2 weeks. Patient was discharged from the hospital after 3 weeks seemingly perfectly well.

In Figure 10,  $S_1$  and  $P_1$  represent respectively sugar and inorganic P changes in the blood sample taken 1 hour after admission. The blood sugar was 336 mgm per cent, the hyperglycemia being due presumably to some meningeal irritation. The curves  $S_2$  and  $P_2$  represent changes in sugar and inorganic P in a blood sample taken 1 week after admission when the boy was quiet with normal temperature. This figure may be compared with Figures 3 to 8 in

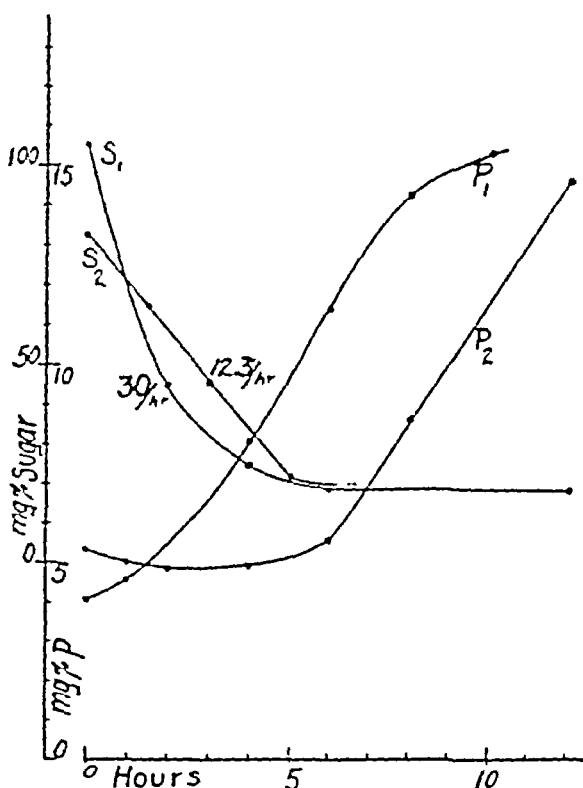


FIG 12 PYELONEPHRITIS, WITH RECOVERY

I S., female, 2 $\frac{1}{2}$  years pyuria and fever,  $S_1$  and  $P_1$ , from blood sample taken at time of admission,  $S_2$  and  $P_2$ , from blood taken after recovery

nonprotein nitrogen was high and coma, convulsions and death ensued a few hours after this sample was taken. The glycolytic rate was normal (14 mgm per cent per hour), but the inorganic P in the blood was 12.6 mgm per cent and increased from the start of incubation.

Case 12, Figure 12 Pyelonephritis, with recovery. I S., female, age 2 $\frac{1}{2}$  years. History of illness of several weeks, with marked pyuria. At the time of admission to the hospital there was marked diminution of urinary secretion. The girl was drowsy and had slight hyperpnea. The blood nonprotein nitrogen was 94 mgm per cent. In Figure 12, the curves  $S_1$  and  $P_1$  represent changes of the sugar and inorganic P in a blood sample taken at the time of admission. In this blood glycolysis was rapid and the inorganic P increased rapidly from

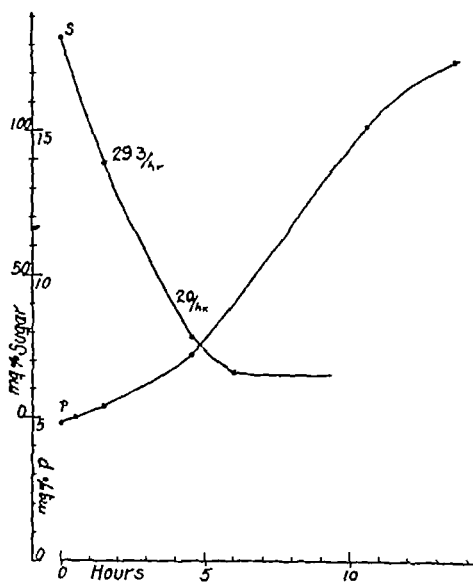


FIG 13 GASTRO INTESTINAL INTOXICATION

E S, female, 2 months, acutely ill

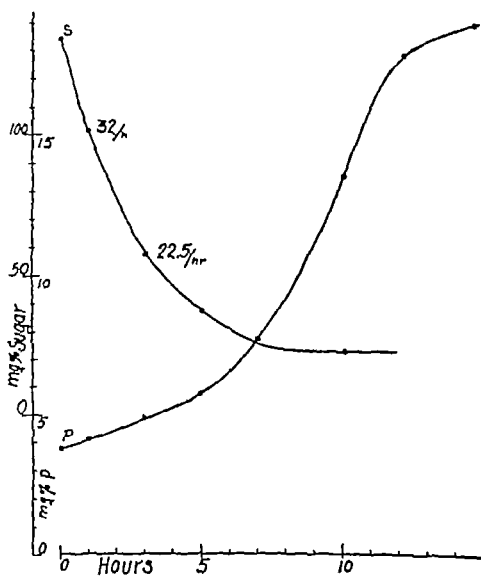


FIG 14 GASTRO-INTESTINAL INTOXICATION

R H, male, 7 months, acutely ill

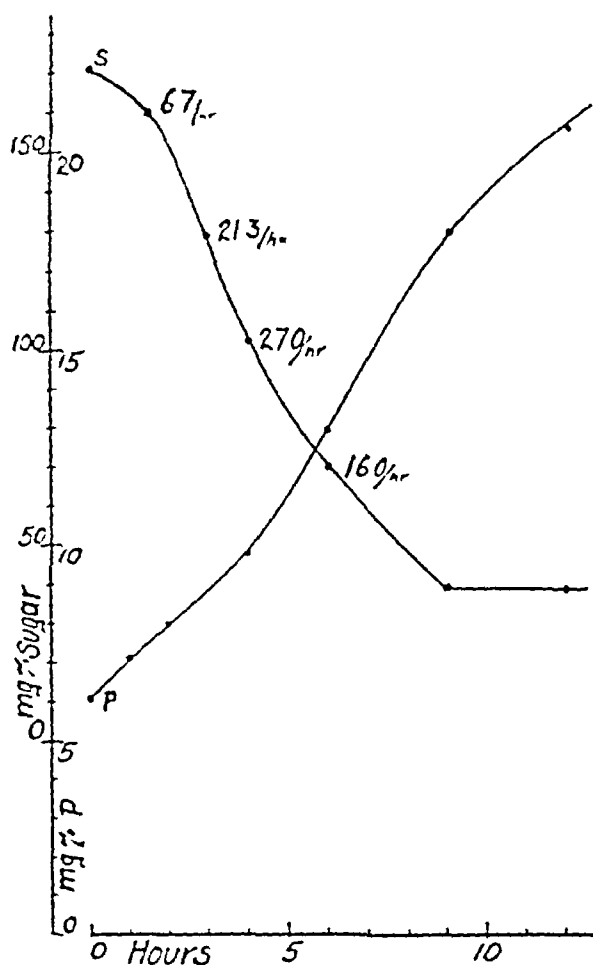


FIG 15 GASTRO-INTESTINAL INTOXICATION  
A E, male, 4 months, acutely ill

Glucose in the blood sample taken at the time of admission was accelerated, 20.3 mgm per cent loss per hour, and the inorganic P rose slowly from the start of incubation of the blood

Case 14, Figure 14, Gastro-intestinal intoxication R H, male, age 7 months, admitted with history of mild diarrhea for 3 weeks, and vomiting during the last 5 days. Weight 16 1/2 pounds. Temperature was 101° F. The

infant was drowsy, and the breathing slow and quiet. There were signs of recent loss of weight. The skin was dry and inelastic, of grayish color. Patient died 5 days after admission. In the blood sample taken at the time of admission the blood glycolysis was accelerated 32 mgm per cent loss per hour, and the inorganic P rose from the start of incubation.

*Case 15 Figure 15 Gastro intestinal intoxication* A E male age 4 months, with history and symptoms similar to the last two infants described but apparently less severe. The sugar and inorganic P curves are from a blood sample taken at the time of admission another blood sample taken 6 days later after the signs of intoxication had disappeared, gave normal curves.

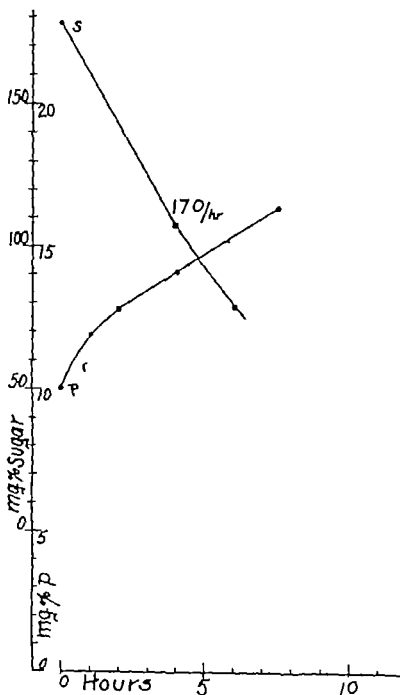


FIG 16 GASTRO INTESTINAL INTOXICATION

E B male, 3 months acutely ill, almost moribund with marked dehydration and acidosis

*Case 16 Figure 16 Gastro intestinal intoxication* E B, male age 3 months admitted to the hospital almost moribund, with a history of marked diarrhea and vomiting of 6 days duration. The infant was markedly dehydrated the eyes sunken the skin dry and inelastic and of dusky gray color. Respirations were deep and labored. Blood nonprotein nitrogen 92 mgm per cent serum

CO<sub>2</sub> content 14 volumes per cent. There was some improvement under treatment for a week, then the infant became worse and died after 3 weeks. In the blood sample taken at the time of admission the glycolytic rate was approximately normal but the inorganic P was 10 mgm per cent and rose sharply from the start of incubation.

#### DISCUSSION

The significance and function of blood glycolysis in the total carbohydrate metabolism of the body is not yet clearly defined. The studies of Warburg and many others have shown that glycolysis has a fundamental importance in the physiology of the tissue cells, constituting part of the respiratory cycle of the cells (Dickens and Simer (1930)), and Warburg (1925) showed that glycolysis serves as a valuable index of the metabolic activity of various tissues. Blood cannot be regarded as an inert carrier, and in view of its bulk it would be surprising if its own metabolic activities were not of considerable importance in the total metabolism of the body. Aside from the possible quantitative importance, however, the mechanism of glycolysis may well have a considerable significance in reflecting something of the nature of the chemical reactions which occur within the tissues.

Variations in the rate of glycolysis may be due to changes in concentration of the enzyme or enzymes responsible for various steps in the process, changes in concentration of the substrate (the blood sugar), changes in concentration or nature of other substances (such as phosphates) which may be essential to these reactions, or changes in chemical factors (such as the acid-base equilibrium, shifts of pH, etc.) which affect and govern the enzyme activities. As stated in the preceding paper, it appears that most of the glycolysis is due to the erythrocytes. Leucocytes also have high glycolytic power, and this might be regarded as very significant since an accelerated glycolytic rate may be observed in most bloods with a high leucocytosis, but Schmitz and Glover (1927) in studying 7 cases of leukemia found that, although the rate was rapid in the blood of these patients, there was no real correlation between the number of white cells and glycolysis. Probably the most important factors responsible for alterations of glycolysis and alterations of the behavior of the blood phosphorus during glycolysis as described here, may be found in changes in the chemical state of the blood which affect the enzyme reactions. Martland (1925) suggested that the high concentration of inorganic phosphorus in the blood when observed in states of acidosis in many diseases might be due to the fact that a shift in the reaction of the blood towards acidity diminishes phosphoric ester synthesis and leaves the inorganic phosphorus increasing in the blood because of ester-hydrolysis. Phosphoric ester hydrolysis occurs over a range of pH 6.0 to 9.0 (Rona and Iwasaki 1927), while ester synthesis is extremely sensitive to such changes, being inhibited by any shift of pH below 7.3 (Martland

(1925)) and stopped below pH 6.8 (This has been discussed briefly in the preceding paper)

When dealing with substances of the blood which are known to be extremely labile, it should be recognized that the values obtained from their measurements are not static for example, a given concentration of blood sugar in a diabetic has a different significance when that sugar is increasing than when it is decreasing. The element of change, and rate of change *in vivo* in such circumstances can be brought out only by repeated blood sugar determinations made at short intervals. The changes in blood constituents which are observed *in vitro* as in glycolysis, may not be identical with changes which occur *in vivo* but by study of such changes at least one set of chemical reactions characteristic of a given sample of blood may be analyzed. The blood of the infant in Figure 16 had an inorganic phosphorus content of 6.1 mgm. per cent, only slightly above the usual value for a normal infant of this age, but this inorganic phosphorus was in a state of rapid increase, due to the splitting of organic phosphorus compounds within the cells. Whatever may be the cause of the increased hydrolysis of the phosphoric esters (or partial failure of their resynthesis) this increased rate of liberation of inorganic phosphate under such circumstances must have a considerable importance in the total phosphorus metabolism and also in the acid base equilibrium of the whole body. Normally the inorganic phosphate in the blood is kept at a fairly constant level, and constitutes only 2.5 mEq. of the total acids (160 mEq.) of the serum. Except in extreme conditions (in uremia, for example) the inorganic phosphorus rarely increases to more than double its normal value a concentration that has been considered rather negligible compared to the increases of other acids observed in severe acidosis due to different causes (Peters, Wakeman, Eisenman and Lee (1929)). The maintenance of the inorganic phosphorus at such a constant level is to a considerable degree a function of the kidneys, and the importance of the urinary excretion of phosphates to the acid base equilibrium of the body is well known. Part of the inorganic phosphorus freed in the blood from both blood and tissue cells, is resynthesized to organic compounds. An increased speed of liberation of inorganic phosphorus must place a considerable burden upon the adjusting mechanisms which are responsible for keeping constant the level of inorganic phosphorus in the blood, and herein may lie the chief significance of the changed pattern of reactions displayed in the figures.

The formation of lactic acid during glycolysis in these bloods was not determined but it has been repeatedly demonstrated by others that in blood glycolysis each molecule of sugar is split to two molecules of lactic acid. Relatively little oxidation of the lactic acid occurs as it is formed during glycolysis *in vitro*, but in the body the lactic acid is caused to disappear from the blood by processes of oxidation and also by its resyn-





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- Warburg O J Cancer Research, 1925 ix 148 The Metabolism of Carcinoma Cells



## INCREASED ELASTIC TENSION OF THE LUNG IN EXPERIMENTAL PNEUMONIA

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A well recognized phenomenon of pneumonia is reduction of respiratory motion on the side of the chest occupied by the lesion. Restriction of costal movement is usually plainly apparent to the bedside observer, and this together with elevation and restriction of the hemidiaphragm may be distinguished by aid of the x-ray (1, 2, 3, 4). Our own observations of these signs in man, which are to be published elsewhere, were assisted considerably by the use of a double exposure roentgenographic method (5) whereby the shadows of the pulmonary environs at inspiration and at expiration were superimposed on the same film for comparison. The ribs and diaphragm on the side of the lesion were shown characteristically to move less than normal and to confine their excursions to the expiratory portions of their normal ranges of motion, even during maximum efforts to inspire. When pneumonia was limited to one lobe, the ribs overlying that part were often more restricted than were those over the normal lobes on the same side. The diaphragmatic dysfunction was usually greater when the lesion was in the lower lobe than when it was in the upper, and was greatest when the entire hemilung was consolidated to some extent. On the other hand, the diaphragm moved normally in many cases when only one lobe, whether the upper or the lower was involved. Sometimes the heart shifted slightly toward the diseased lung at inspiration, while it occupied a normal position at expiration.

Three causes have generally been assigned for the abnormalities of respiratory movement in pneumonia, namely, reflex spasm or inhibition of the respiratory muscles, blockage of the alveoli and ducts with inflammatory exudate, and collapse of the alveoli. In the presence of sharp pleuritic pain the intercostal muscles become obviously spastic from both voluntary and involuntary protective reactions but without the pain, although breathing movements are greatly reduced on the side of the lesion, spasticity is less well defined or cannot be demonstrated at all. A certain amount of evidence is available to suggest that under the latter conditions the muscles are inhibited. Heuer and Holman (6) and others (7) have shown that artificial stimulation of the central stump of a

divided vagus nerve in animals causes the diaphragm temporarily to cease moving and to assume the elevated position of complete relaxation, and they interpret the effects as due to inhibition of the phrenic nervous centers. Furthermore, Newburgh, Means and Porter (8) found that in pneumonia the vagi carry centripetal impulses which act upon the respiratory centers to render the respirations shallow and rapid. It may well be, therefore, that the reduction of costal and diaphragmatic excursions which is under discussion is produced by inhibitive impulses from the lungs (2). The second and third causes for the abnormalities of breathing are perhaps more manifest than the first, since with the alveoli rendered unavailable for reception of air, either because they are filled with exudate or collapsed, that part of the lung cannot expand at inspiration and probably impedes the expansion of the adjacent region of the thoracic parietes. A common observation at autopsy is that consolidated areas in pneumonic lungs expand poorly or not at all when the lungs are excised and then inflated by blowing into the trachea (9, 10). The space normally intended for air is occupied by fluids. Coryllos (10) believes that atelectasis occurring in the early stages of pneumonia is chiefly responsible for the changes in position and motion of the breathing parts.

The changes in breathing in our clinical cases were not always easily explained by these hypotheses, because they were often found before any consolidation of the lung was demonstrable and remained for some time after consolidation and all outward signs of pulmonary irritation had gone. In one instance elevation and confinement of the diaphragm persisted for more than three weeks after complete reaeration of the lung had occurred and cough had disappeared. Reflex nervous inhibition could account for the changes during the preconsolidative stage, for the lungs were then distinctly catarrhal, but that seemed probably not to be the cause for them late in the recovery period. An hypothesis hitherto unconsidered presented itself, that alterations in elasticity of the lung parenchyma occurred at these stages of the disease and were chiefly responsible. This seemed the more likely because soft tissues in other parts of the body become stiffer than normal during, and for a period after, the presence of acute inflammation, and because increased elastic tension of the pulmonary alveolar and lobular septa at the pre- and post-consolidative periods of pneumonia would be expected to increase the resistance to pulmonary inflation and impede the excursions of the thoracic parietes.

The elasticity of the lungs has been the subject of much investigation. Perhaps the best work on the normal lungs is that of Cloetta (11). As to the pathological changes in this property, interest has centered principally in the loss of tension in emphysema (12, 13, 14). The statement that the elastic tension is increased in pneumonia appears here and there

in the literature (15), but few writers have made direct measurements to prove the truth of the assertion Perls (14), in 1869, attached a manometer to the trachea of human subjects dead of various diseases, then opened the chest and read the manometric pressure produced by the recoil of the lungs. He found greater pressures in cases with pneumonic consolidation than in those with normal lungs. However, he was not satisfied with the results because of the postmortem changes that had occurred before autopsy, and others (14) have not accepted them for the same reason. Coryllos states, and it is our experience too, that human lungs at autopsy frequently leak air from the pleural surfaces on inflation so freely as to interfere with the results of such experiments. Bittorf and Forschbach (16) insufflated the trachea of human subjects with air after death without opening the chest and measured the pressure necessary to expand the chest. They found increased pressures in the presence of pneumonic consolidation. Tendeloo, Hennemann and Metz (12) tested strips of tissue excised from the cortex of lungs of man. The length of each strip was measured, the strip was stretched momentarily to a certain degree, and the length after relaxation was determined. The degree of approach to the initial length was used as the index of elasticity. The specimens from consolidated lungs proved considerably less elastic than those from normal lungs. Apparently only Joannides and Steinman (17) have paid attention to the nonconsolidative stages of pneumonia. They reported from microscopic study of the living dog's lung that the alveolar walls were thicker and seemed to stretch at inspiration less easily than normal when pneumonia was incipient and the air spaces and passages were still patent.

The experiments to be described were designed to determine from freshly excised lungs of dogs to what degree the pulmonary tissues develop increased elastic tension in pneumonia, especially before and after the period of consolidation, and how much this affects the gross pulmonary expansion. Dogs were chosen as subjects because they are known, after proper intrapulmonary inoculation, to run a course of pneumonia that closely resembles the spontaneous lobar pneumonia in man.

#### EXPERIMENTAL MATERIALS AND METHODS

Pneumonia was produced in 13 dogs by the method of Terrell, Robertson and Coggeshall (18-19) with the modification that inoculation was done with the aid of bronchoscopy. The bronchi of the right lower lobe were examined by x-ray from day to day. Three were sacrificed at what seemed to be the height of pneumonic consolidation, 3 to 7 days after inoculation, and the remaining five were sacrificed 2 to 6 days after disappearance of consolidation which was 14 to 17 days after inoculation.<sup>1</sup> The animals were electrocuted

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<sup>1</sup> As a rule within 24 hours after inoculation typical consolidation of the injected lobe has occurred. The disease runs a febrile course of 3 to 7 days, the pneumonic lesion either remains localized in one lobe or spreads from lobe

to avoid the agonal changes frequently produced in the lungs by other methods of killing. Autopsy was begun by exposing and clamping the trachea in the neck. The chest was opened and the lungs were examined, special note being made of the relative sizes of the lower lobes, inflated as they then were to the degree of passive expiration.\* All vessels supplying the lower lobes were ligated to retain the fluids, and the lungs were removed *en masse* from the

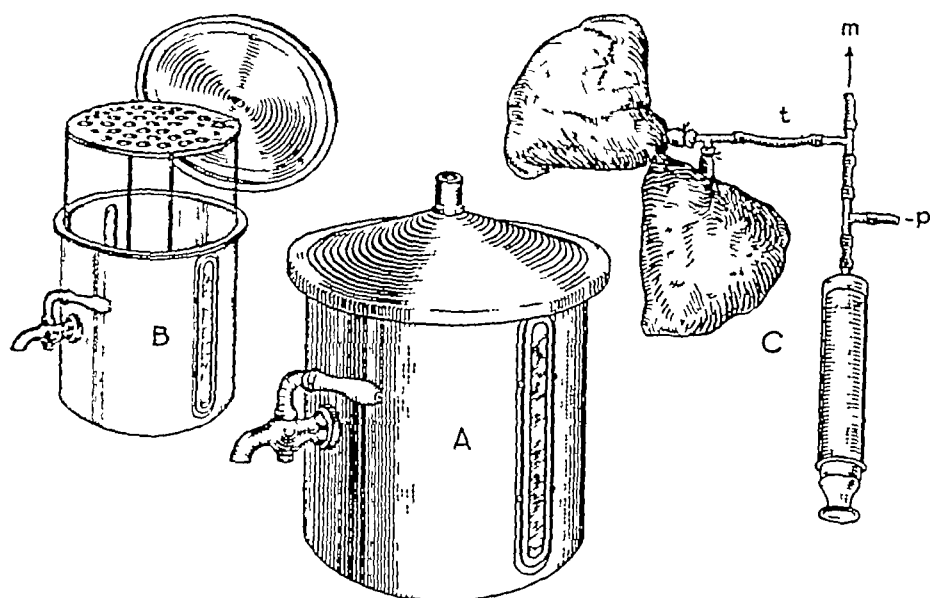


FIG 1 APPARATUS FOR INFLATING EXCISED PULMONARY LOBES WITH EQUAL, MEASURED PRESSURES OF AIR AND DETERMINING THEIR VOLUMES

The volumetric chamber is represented at *A*. It is shown again at *B* with cover and platform lifted for insertion of a lobe. At *C* are two lower lung lobes attached to the same system of tubes and syringe for inflation. The manometer which is connected at *m* is not shown. The tube at *p* is opened for admission of air to the syringe, and the tube at *t* is opened only when the tube at *p* is closed for injection of the air into the specimens.

to lobe. This experimental disease resembles the natural disease in humans in the manner of spread of the lesion, the localization of the process, the immune response, the abrupt termination of the disease by crisis, lysis, or death, and the rapid regression of the process after recovery. With the evolution of the disease the lesion progresses through the different stages observed in the human pneumonic lung. " (18)

Many more dogs were inoculated than those enumerated above, but they were discarded, either because x-ray examination during life or after death showed pneumonia in the left lower lobe or only abortive pneumonia in the right lower lobe or because the lobes were accidentally punctured at the time of removal and leaked air on reinflation.

\* Attempts were made in the earlier experiments to measure accurately the volume of each of the lower lobes in this state of inflation, but without success because the handling of the lungs incident to their removal and preparation for such measurement necessarily caused displacement of air from lobe to lobe and marked changes in size of the lobes.

chest. The stem bronchi of all but the two lower lobes were now ligated and then divided peripheral to the ligature which left the primary bronchi airtight and only the lower lobes attached. The clamp on the trachea was removed, the lobes were allowed to collapse, and a photograph and a roentgenogram were made. Each primary bronchus was then ligated near its origin at the trachea and was divided between the ligature and the trachea.

The volume of each lobe was obtained by immersion in water in a specially constructed chamber (Fig 1 *A* and *B*). This was a cylindrical metal vessel fitted with a spigot and covered with a conical plate that was ground to fit the top accurately. The cover was perforated at the peak by a short tube. A removable platform was contained in the vessel. The chamber was made ready for use by greasing the margin of the cover, fitting it in place and filling the chamber with water until the meniscus appeared at the top of the tube in the cover. The spigot was next opened and sufficient water was let out to enable the cover to be removed and one of the lobes to be introduced under the platform without spilling any water. The cover was replaced and the water that had been let out was poured through the tube back into the chamber until

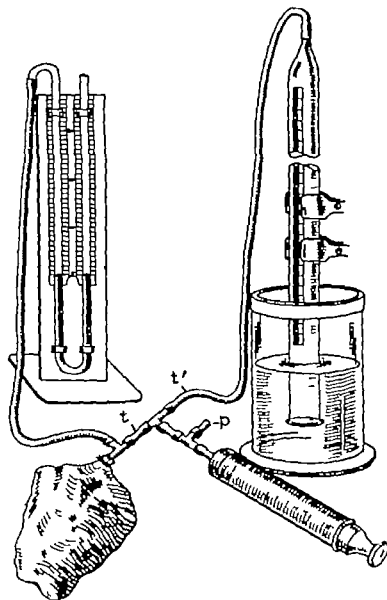


FIG 2 APPARATUS FOR INFLATING AN EXCISED LOBE WITH A MEASURED AMOUNT OF AIR AND DETERMINING THE INTRAPULMONARY PRESSURE

The tube at *p* is opened only to adjust the water level in the burette to the lowest mark on the scale and then closed. The tubes at *t* and *t'* are opened and closed as necessary while pumping air from the burette into the specimen.



the meniscus appeared again at the top. The volume of water left over was taken as the volume of the lobe. After measuring both specimens in this way, the ligatures were removed from the bronchi and the bronchi were applied and held with ligatures on two limbs of a T-shaped cannula. The other limb of the cannula was connected with rubber tubes to a water manometer and a syringe (Fig 1, C) and the specimens were simultaneously inflated with air from the syringe until all parts of the left (normal) lobe were inflated to an extent that was judged to represent natural, moderately full inspiration. The pressure was then adjusted to 10 cm H<sub>2</sub>O by removing a little air. Each bronchus was ligated right next to the cannula, to maintain the inflation, and was freed from the cannula. Once more each lobe was measured volumetrically, photographed and roentgenographed.

After this the ligatures were taken off, the air was allowed to escape, and the bronchus of the right (pneumonic) lobe alone was cannulated and was connected to a water manometer, a syringe, and a burette which had been inverted and placed with the lower end in a dish of water (Fig 2). The water level in the burette was adjusted to the lowest mark on the scale, and then air was drawn from the burette by the syringe and deposited in the lobe until the nonconsolidated portions of the lobe were fully inflated.<sup>3</sup> The amount of air required to do this and the manometric pressure were noted. The lobe was replaced by the other and the test was repeated, this time, however, using only the amount of air injected into the first lobe.

Finally, the lobes were sectioned at numerous places, including the most consolidated and most air-containing regions, for gross and microscopic examination.

The entire procedure, except for the inoculation, was applied to 5 other dogs, to obtain normal controls.

#### RESULTS

The data thus obtained for each lobe of every pair included (1) the relative size at natural expiration, (2) the absolute size at complete collapse, (3) the absolute size at inflation with equal pressures as at natural inspiration, (4) the pressure at inflation with equal amounts of air, and (5) the gross, microscopic and roentgenographic appearances of the tissues. The measurements from the four groups of dogs are given in the tables.

(1) *Size at expiration* The two lobes appeared to be very nearly, if not quite, the same size, whether pneumonia was present or not and whatever was the stage of the lesion.

(2) *Size at collapse* In the control dogs the two lobes showed very little difference in size, the greatest difference being 7 per cent. In 3 of them the right lobe was the larger, and in 2 the left was the larger. At the preconsolidative stage of pneumonia the right lobe was larger by 6 to 11 per cent, at the consolidative stage the right was larger by 2 to 34 per cent, and at the postconsolidative stage the right was the same size as the left in 1 case, 2 per cent smaller in 1 case, and 3 to 9 per cent larger in 3 cases (Figure 4).

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<sup>3</sup> Attempt was not made to equalize the water-levels of the burette before taking the manometric reading, because the difference of level was exactly the same with the two lobes and we were interested only in comparative pressures.

(3) *Size at inspiration* The lobes from the control dogs had slight differences in size, the greatest difference being 8 per cent. In 2 of them the right was the larger and in 3 the left was the larger. At the preconsolidative stage the left lobe was larger by 13 to 48 per cent, at the consolidative stage the left was larger by 40 to 60 per cent, and at the post-consolidative stage the left was larger by 19 to 39 per cent. (Figure 4)

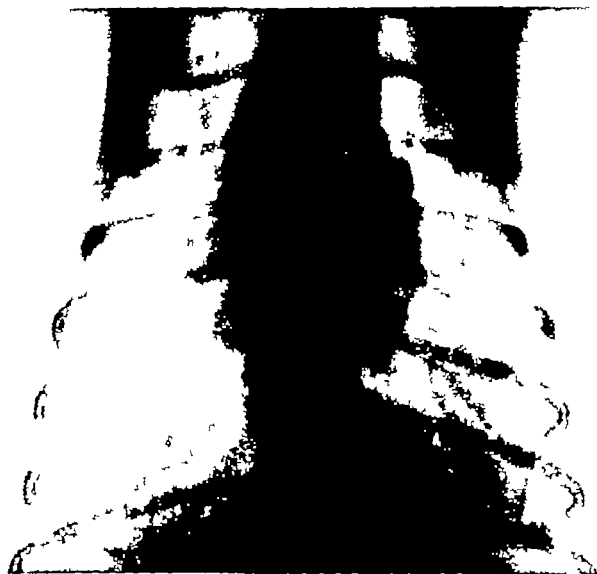


FIG 3 ROENTGENOGRAPHIC APPEARANCE OF A DOG'S CHEST 3 DAYS AFTER INOCULATION OF THE RIGHT LOWER LOBE WITH PNEUMOCOCCUS

The lobe (on the right) is seen to be completely consolidated and the right hemidiaphragm to be slightly elevated

(4) *Pressure at inspiration* The lobes from the control dogs exhibited a fair degree of uniformity of pressure, the greatest difference being 16 per cent. In 1 of them the pressure was the same, in 2 that of the right was the greater and in 2 that of the left was the greater. At the preconsolidative stage of pneumonia the pressure of the right lobe was greater by 23 to 38 per cent, at the consolidative stage that of the right was greater by 42 to 75 per cent, and at the recovery stage that of the right was greater by 11 to 43 per cent.

(5) *Appearance* The lobes of the control dogs had no significant alterations

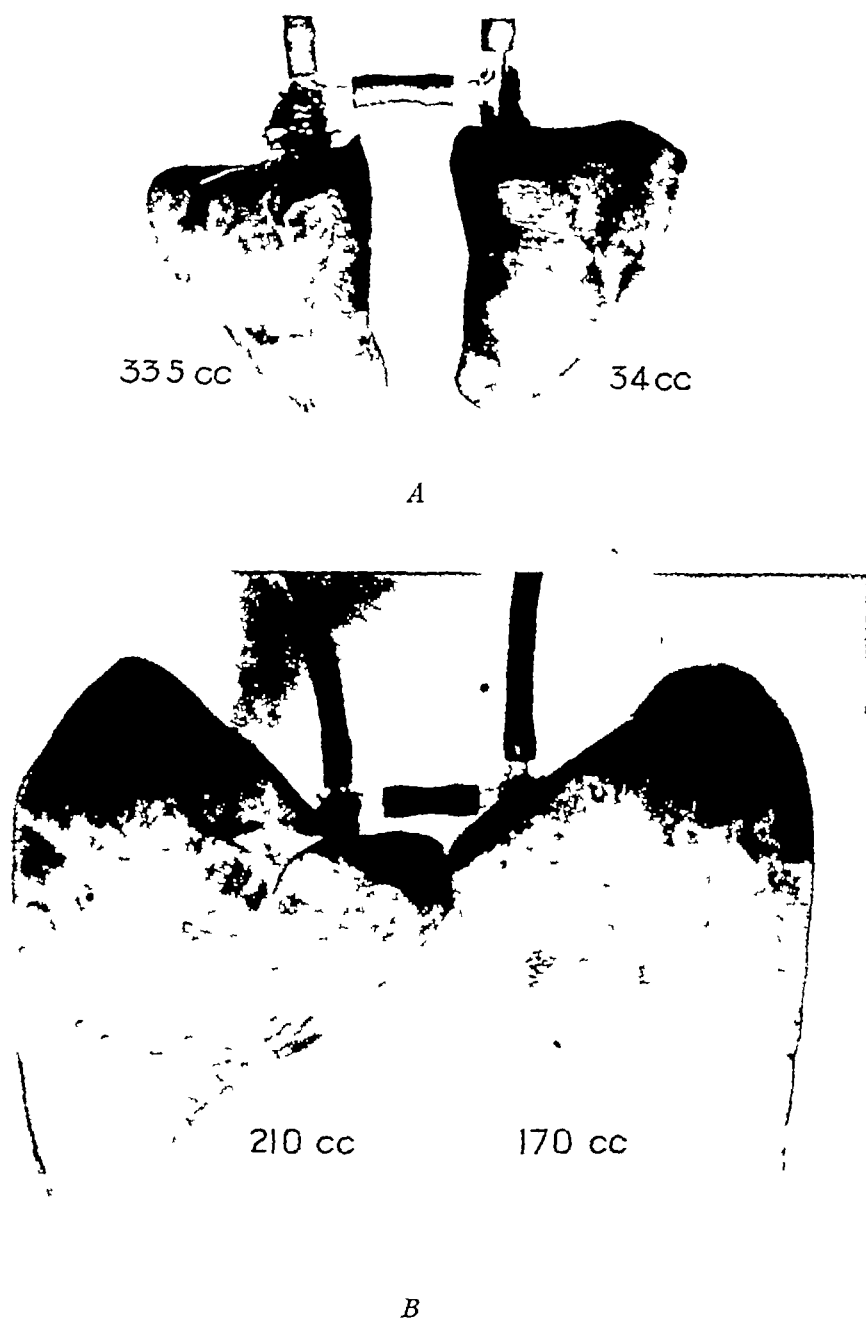


FIG 4 THE TWO LOWER LOBES OF THE DOG OF FIGURE 3, REMOVED 17 DAYS AFTER INOCULATION AND 7 DAYS AFTER DISAPPEARANCE OF CONSOLIDATION

The lobes are collapsed at *A* and expanded at *B*, and the corresponding volumes are inscribed. Note the lack of consolidation in both lobes, the approximate equality of size at collapse, and the smaller size of the inoculated lobe (on the right in each view) at expansion.

Both lobes of the inoculated dogs presented gross changes at the pre consolidative stage, the right however, much more than the left. The right lobe was redder than normal throughout, particularly along the medial border and about the hilus. Numerous petechiae were scattered over the surface at various points, also more especially at the medial border and hilus. The tissues were everywhere crepitant and seemed slightly heavier than normal. Section showed the parenchyma to be red and wet in those regions with the greater surface changes, and to be practically normal in other parts. No consolidation was discernible. The left lower lobe presented slight superficial redness limited to the medial aspect of the hilic region and redness and wetness of the parenchyma beneath, but it was normal elsewhere. The peribronchial connective tissues and lymph nodes of both lobes were somewhat edematous. Microscopic examination revealed an air containing condition throughout in both lobes, except for a few small areas in the cortex of the right lobe at the hilic region where a few of the alveoli were filled with erythrocytes and edema fluid. The capillaries and smaller arterioles and venules were dilated with blood in many parts of the right lobe but only in the hilic region of the left lobe. In these places the alveolar walls were two or three times the normal thickness, due to the presence of dilated capillaries increased interstitial fluids and a few lymphocytes. The alveolar sacs were contracted to one-third or one fourth the normal size in most instances. In other parts of both lobes there was no alteration. The bronchi and bronchioles were empty.

At the consolidative stage, the right lobe was one-eighth to four fifths airless. The solid portions were sharply demarcated and occupied principally the hilus and body of the lobe, while the air containing parts were mostly at the periphery. The former were swollen and dark purplish red with smooth lusterless surfaces. Section presented a bulging, dry and granular surface in some parts of the consolidated parenchyma, a concave, dry and smooth surface in others, and a moist and somewhat crepitant zone at the periphery, while it showed the air-containing tissues to be without gross abnormality save for slight redness and wetness of the cut surfaces. The left lobe was unaltered grossly except for a little redness and wetness of the tissues near the hilus. The peribronchial tissues and lymph nodes of both lobes were hugely swollen and edematous. Microscopic study of the right lobe showed wide areas of pneumonic consolidation beside narrower ones of atelectasis. The vessels were all markedly engorged and the bronchial passages, large and small, were choked with polymorphonuclear leukocytes erythrocytes, epithelial debris and fluid. Sections from other regions showed the alveoli and bronchi free from exudate. Here, for the most part, the alveolar and lobular septa were distinctly thickened and the alveolar spaces were correspondingly narrowed. Again the septal thickening was due to cap

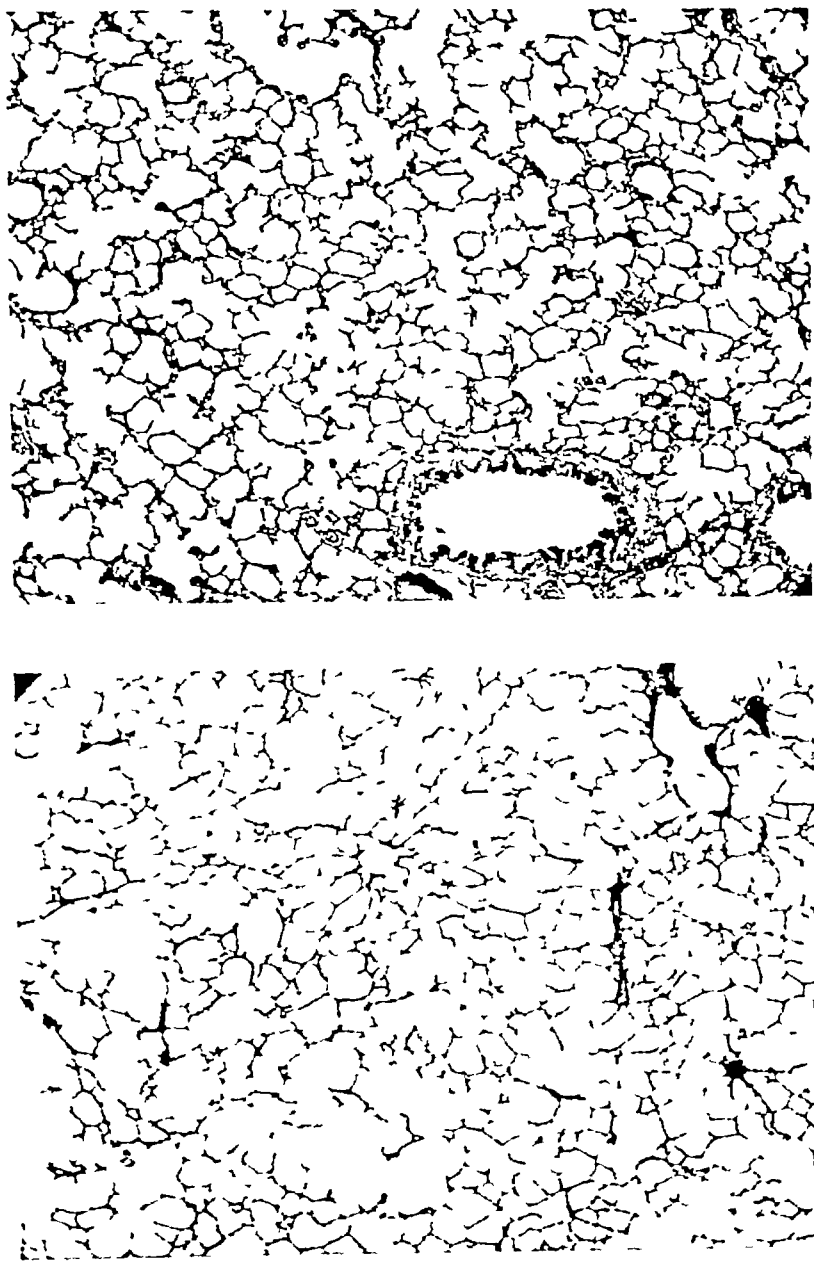


FIG 5 MICROSCOPIC APPEARANCES OF THE LOBES OF FIGURE 4  
The inoculated lobe (on the right) shows alveolar and bronchial patency but thickening of the alveolar septa. The uninoculated lobe shows no abnormalities

illary engorgement and interstitial accumulation of wandering cells and fluid. The left lobe presented no histological changes except in sections from the hilic region where the vessels were engorged and the alveolar septa were slightly thickened.

All animals sacrificed at the postconsolidative stage exhibited at x-ray examination during the first week of the infection complete consolidation of the right lower lobe. At autopsy, however, there was extremely slight gross abnormality of the lungs. This appeared only in two cases and was confined to the inoculated lobe. Near the hilus were small red and wet, but crepitant, patches. Otherwise the right lobe appeared normal (Figure 3). The peribronchial tissues on the right were moderately edematous, and the lymph nodes in this neighborhood and throughout the mediastinum were markedly enlarged and succulent. Fine brick-red granules lay scattered abundantly in the mediastinal and pericardial membranes. Histologically the parenchyma of both lower lobes was completely air-containing, except for an occasional alveolus or small group of alveoli situated near the pleura in the right lobe which was filled with clear fluid or was collapsed. The capillaries and small vessels in all parts of this lobe were slightly dilated, the interstitial fluids were slightly increased, and this caused the alveolar and lobular septa in most places to be distinctly thickened. The septal change was the chief characteristic of the right lobe, and it was in most cases entirely absent in the left lobe (Figure 5). The pleural membrane of the right lobe was also definitely thickened, due chiefly to proliferation of fibroblasts and somewhat to increase of interstitial fluids and cells.

#### CONCLUSIONS

(1) The elastic tension of the dog's lung becomes markedly increased in pneumonia within a few hours after entrance of the organisms, before blockage of the air passages and spaces with inflammatory exudate, also, for several days after reinflation of the parenchyma. No conclusion is drawn as to the elastic tension during consolidation, since exudate in the bronchi and alveoli interferes with the inflation required for the tests.

(2) The increase of elastic tension is probably due to thickening of the alveolar and lobular septa and, in the postconsolidative stage, also to thickening of the pleura.

(3) The size of the lung is changed in pneumonia. In the preconsolidative period, the infected lobe is larger than normal; when fully collapsed, about normal in size when inflated at expiration, and smaller

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\* 'Normal size,' with reference to the right lobe, refers to the size of the left lobe, rather than to the absolutely normal size of the right lobe. This basis of comparison is the more pertinent of the two for the object of the experiments. The lower lobes of the dog lent themselves well for comparison since they were normally very similar in size. See Table 1.

than normal when inflated at inspiration. In the consolidative period, the lobe is larger than normal at collapse, about normal at expiration, and smaller than normal at inspiration. In the postconsolidative period, the lobe is about normal in size at collapse and at expiration, and it is smaller than normal at inspiration.

(4) Increased elastic tension of the parenchyma is probably chiefly responsible for the decreased size of the right lobe at inspiration during the pre- and postconsolidative periods. Increased content of inflammatory products of the lobe is evidently responsible for the increased size at collapse during the preconsolidative and consolidative periods. Blockage of the air passages and spaces with inflammatory products and foci of atelectasis (for the airless parts) and increased elastic tension of the tissues (for the air-containing parts) probably act together to cause the decrease in size at inspiration during the consolidative period.

(5) Without further study<sup>5</sup> it cannot be said how much this increased elastic tension interferes with respiration during life, but judging from the magnitude of the effects on the distensibility and size of the excised lobe which it causes, it may well be expected to curtail the respiratory movements of the ribs and hemidiaphragm adjacent to the inflamed part and to displace the mediastinum, to the extent to which these changes occur in clinical pneumonia.

(6) Since the lung shows unmistakable evidences of irritation throughout the period of experimental observation, it is possible that reflex nervous inhibition, as referred to in the introduction, occurs and contributes to the curtailment of respiratory movements in the pre- and postconsolidative stages of pneumonia, as well as in the consolidative stage.

#### COMMENT

Our histological findings of interstitial cellular infiltration in the early, preconsolidative stage of pneumonia correspond with those of Blake and Cecil (20) in their research on pneumonia in monkeys. They showed that after entrance to the large bronchi, the pneumococci quickly penetrate the bronchial walls and travel to the periphery in the peribronchial lymphatics and connective tissues, producing their first parenchymatous lesions in those tissues. Exudation into the bronchi and alveoli with consolidation followed that process.

It was said in the introduction that patients with inextensive pneumonia occasionally showed no detectable alterations of position and motion of the pulmonary environs. Considering increased elastic tension of the lung as the cause of the alterations, this is not surprising, for the powerful action of the respiratory muscles would be expected completely to overcome small increases in elasticity, at least as far as their extrinsic influences are concerned.

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<sup>5</sup> This study is now in progress.

Whether or not extrinsic effects develop, certain intrinsic ones would be expected to do so, in all stages of pneumonia and in all extents of that disease short of total involvement of the hemilung. Since each half of the thorax is a single chamber filled with elastic pulmonary tissues and without partitions to prevent internal spatial rearrangements from taking place, the relatively tense inflamed parts of the lung should expand at inspiration less, and the uninflamed parts more, than normal. This would shift some of the work of breathing from the former to the latter tissues. During the consolidative stage of extensive subtotal pneumonia of the hemilung this shift occurs and is complete, as is well known, so that the alveoli of the uninflamed part become greatly over expanded and emphysematous, which is detectable during life by physical signs (Skodaic resonance, etc.) and after death by persistence of dilated alveoli in the collapsed specimen as viewed at autopsy. But in the consolidative stage of inextensive pneumonia where a wide field of normal hemilung remains to take over the function, and in the pre- and postconsolidative stages of inextensive or moderately extensive pneumonia where the functional shift is partial, emphysema of detectable proportions would not be expected to develop and the reapportionment of function that takes place might well go unnoticed. Whether the effect of increased elastic tension is purely intrinsic or both in- and extrinsic, it is obviously an agent of rest for the inflamed tissues, which operates automatically from a very early to a very late period of the disease. It would be expected to act whether or not the inflammation progressed to consolidation.

The statement of the older pathologists that the pneumonic lung in man is larger than normal is based upon comparison at autopsy of the consolidated lobe with the collapsed, normal lobes. This, of course, agrees with our findings in dogs.

The belief of Coryllos that the smaller size of the pneumonic lung at inspiration is due to the presence of atelectasis may be true in the early consolidative stage of which he writes, as far as these data go, but at the pre- and postconsolidative stages it is clearly not due to atelectasis.

The elastic tension of the lung is known to become increased in another kind of pulmonary disease, namely, tuberculosis. There it is often brought strikingly to one's attention when artificial pneumothorax or phrenic paralysis is induced, for then selective collapse and healing of the diseased segment of lung may occur. This subject has been discussed at length recently by one of us (21). It seems probable that the increased tension that is responsible for selective therapeutic collapse in this disease is due in some part, too, to thickening of the pulmonary septa and membranes from dilatation of vessels and interstitial deposits of inflammatory fluids and cells.



TABLE 1

*Observations on both lower lobes of normal control dogs' lungs*

Dog number	Lower lobes	Volumes of lobes when			Pressures in lobes when inflated by equal volumes of air
		Collapsed	Inflated by equal pressures of air		
		cc	cc.	per cent increase	cm H <sub>2</sub> O
1	R	51	274	437	19
	L	50	275	450	19
2	R	36	237	558	24
	L	38	255	571	20
3	R	37	222	500	28
	L	40	237	492	25
4	R	57	331	480	22
	L	55	315	472	24
5	R	43	268	523	20
	L	42	265	531	22

TABLE 2

*Observations on both lower lobes of dogs' lungs at the preconsolidative stage of pneumonia in the right lower lobe, 6 hours after inoculation*

Dog number	Lower lobes	Volumes of lobes when			Pressures in lobes when inflated by equal volumes of air
		Collapsed	Inflated by equal pressures of air		
		<i>cc.</i>	<i>cc.</i>	<i>per cent increase</i>	<i>cm H<sub>2</sub>O</i>
1	R	24	137	471	26
	L	22	188	755	19
2	R	37	155	318	24
	L	33	230	597	18
3	R	30	153	410	27
	L	28	180	543	20
4	R	44	243	452	26
	L	40	280	600	16
5	R	49	202	312	26
	L	46	265	476	20

## SUMMARY

The fact is recalled that in clinical pneumonia may occur reduction of costal and diaphragmatic movements on the side of the lesion, together with lateral displacement of the mediastinum. The causes commonly

given for this are enumerated and discussed, and it is pointed out that they explain the phenomena well in the consolidative stage of pneumonia but not well in the pre and postconsolidative stages. The hypothesis is advanced that the phenomena in the latter stages may be due to increase in the elastic tension of the inflamed tissues.

Experiments with dogs are described which show that inflamed pulmonary lobes at the pre- and postconsolidative stages of pneumonia possess markedly increased elastic tension, as indicated by reduction in

TABLE 3

*Observations on both lower lobes of dogs' lungs at the consolidative stage of pneumonia in the right lower lobe, 3 to 7 days after inoculation*

Dog number	Lower lobes	Fractions of lobes consolidated	Volumes of lobes when			Pressures in lobes when inflated by equal volumes of air
			Collapsed	Inflated by equal pressures of air		
			cc.	cc.	per cent increase	cm H <sub>2</sub> O
1	R	4/5	44	87	197	28
	L	0	29	146	503	8
2	R	4/5	49	122	249	24
	L	0	48	250	521	6
3	R	1/8	32	106	331	24
	L	0	30	272	907	14

TABLE 4

*Observations on both lower lobes of dogs' lungs at the postconsolidative stage of pneumonia in the right lower lobe, 14 to 17 days after inoculation*

Dog number	Lower lobes	Volumes of lobes when			Pressures in lobes when inflated by equal volumes of air
		Collapsed	Inflated by equal pressures of air		
		cc.	cc.	per cent increase	cm. H <sub>2</sub> O
1	R	34	170	400	19
	L	34	210	518	17
2	R	45	214	375	28
	L	41	324	690	16
3	R	40	210	425	26
	L	41	342	733	19
4	R	32	134	318	25
	L	31	180	480	19
5	R	36	192	433	24
	L	34	256	653	16

size and elevation of the intrapulmonary pressure at inflation of the lobes. The increased tension appears to be due to thickening of the alveolar and lobular septa and pleural membranes from dilated capillaries and interstitial inflammatory fluids and cells. The changes of elasticity are great enough to account in considerable part at least for the alterations in movement and position of the pulmonary environs that occur clinically in pneumonia.

The change in elasticity is believed to have the effect also of shifting some of the work of breathing from the inflamed to the normal parts of the lung. The change is believed to be very similar to that which occurs in certain cases of pulmonary tuberculosis and which is responsible for selective collapse of the lung after pneumothorax and phrenicotomy.

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## THE EXCRETION OF INORGANIC SULPHATES

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Among many estimations of blood concentration and rate of excretion of various substances in the urine, relatively few have been concerned with inorganic sulphates. These, regarded as waste products highly concentrated in the urine, are usually classed with creatinine and many foreign bodies as "no-threshold" substances. Cushny (1926), however, cautioned that "sulphates cannot yet be assigned a definite position as it is uncertain whether they should be placed along with phosphates (low threshold) or among the no threshold bodies."

After injection of sulphate and creatinine in rabbits, Mayrs (1922) did not find them concentrated by the kidney to exactly the same degree, although the ratios were so close he regarded it as strong evidence that they were excreted by the same process. The slight differences in the concentration ratios he attributed to reabsorption from a glomerular filtrate of small amounts of even these substances during its passage down the tubule. Poulsson (1930a) found the concentration in milligrams per cent of inorganic sulphates in human urine proportional to the degree of concentration of the urine estimated by urine and blood creatinine, concluding that the two substances were eliminated by the same process and concentrated to the same degree, i.e., that sulphates had no threshold, or that little if any diffused back through tubule cells. Ambard (1931), likewise, grouped sulphates with the no-threshold bodies.

White (1923) on the other hand did not find any regular correspondence in concentration ratios of phosphate, sulphate and sugar in phloridzinated dogs and this led him to exclude these from "no threshold" bodies and to fall back on tubular secretion of sulphate.

Marshall and Crane (1922) found the excretion of sulphate increased by section of the splanchnic nerve, while that of creatinine was little affected. They suggested that possibly some sulphate may be reabsorbed from the glomerular filtrate, or that additional sulphate may be secreted by the tubule cells.

The studies here described were undertaken with the thought of checking the estimated volume of glomerular filtrate according to Rehberg's (1926) creatinine technique by simultaneous determinations of

Serum $\text{SO}_4$	Ultrafiltrate $\text{SO}_4$
<i>mgm per cent</i>	<i>mgm per cent of <math>\text{SO}_4</math></i>
4.4	4.6
4.5	4.5
4.8	5.0

These results led us to believe that we could estimate serum sulphates with sufficient accuracy for the present study.

Most of the subjects were hospital patients without evidence of kidney disease, at rest in bed in the postabsorptive state. The remainder were healthy staff members engaged in light laboratory work during the experiment. An hour after the ingestion of 3 to 7 grams of creatinine the bladder was emptied, and the urine discarded. After an accurately measured time interval, usually an hour, the bladder was again emptied as completely as possible. Blood samples were obtained at the beginning and end of the period of urine collection, allowed to clot and serum separated after centrifuging. All analyses were made on serum. Creatinine was estimated in each sample, the average being assumed to represent the blood level during the period of collection. The same procedure was followed for urea nitrogen in most instances, in a few experiments pooled serum was used. Sulphates were estimated in pooled samples except after injection of sulphates, when of course blood collected at beginning and end of period of urine collection was analysed separately. All analyses were made in duplicate, sulphates usually in triplicate. The analytical methods employed were: serum sulphates by Power and Wakefield's method (1931), urine sulphates by Fiske's (1921) benzidine method after removal of phosphate and Folin's (1905) gravimetric technique, creatinine in serum and urine by Holten and Rehberg's (1931) modification of Folin's method, using a Burkner colorimeter, urea nitrogen in serum and urine by Van Slyke's (1927) manometric method. Known solutions were analyzed at frequent intervals.

After intravenous injection of sulphate, the rate of excretion per minute is proportional to the blood concentration (Table III, Figure 1). The amount of sulphate injected varied from 10–30 grams of  $\text{Na}_2\text{SO}_4 \cdot 10 \text{H}_2\text{O}$ . This was dissolved in 50–150 cc of distilled water, autoclaved, and injected slowly by gravity or syringe during a period of 15–45 minutes. The subject experienced no discomfort, and no reaction of any kind was detected. Five to ten minutes after the end of the injection, the bladder was emptied and the first blood sample obtained from the opposite arm immediately afterward. An hour later, the first sample of urine and another blood specimen were obtained. From one to four collections of urine, and two to five blood samples were taken in different experiments. The diuresis produced by these injections was only moderate—not as great as that following the ingestion of 1.5 to 2.0 liters of water. The data presented in Table III are from five experiments on two subjects.

TABLE III

*Relation of inorganic sulphate excretion and serum inorganic sulphate*

Serum SO <sub>4</sub>	Urine SO <sub>4</sub>	Urine volume	SO <sub>4</sub> excreted	Concentration ratio SO <sub>4</sub>	Concentration ratio creatinine
<i>mgm. per 100 cc.</i>	<i>mgm. per 100 cc.</i>	<i>cc. per minute</i>	<i>mgm. per minute</i>		
*4.3	7.4	19.40	1.5	1.7	10.0
*4.3	192.0	1.61	3.1	44.6	68.0
10.8	434.0	3.85	16.7	40.0	46.0
15.1	359.0	6.18	22.2	23.8	27.4
19.2	309.0	6.49	20.0	16.1	21.9
19.3	746.0	2.17	16.2	38.6	77.0
22.9	745.0	3.37	25.1	32.5	35.4
23.4	344.0	8.95	30.7	14.7	19.7
38.1	1352.0	2.90	39.2	35.5	46.2
43.4	672.0	8.0	53.8	15.5	24.3

\* No sulphate injected

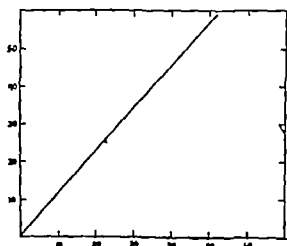


FIG 1

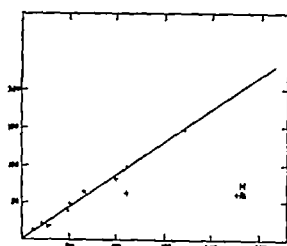


FIG 2

FIG 1 RELATION OF INORGANIC SULPHATE EXCRETION AND CONCENTRATION OF INORGANIC SULPHATE IN SERUM

Abscissae, serum inorganic sulphates, milligrams per cent SO<sub>4</sub> Ordinates, milligrams SO<sub>4</sub> excreted per minute

FIG 2 RELATION OF CONCENTRATION OF INORGANIC SULPHATES IN URINE, AND DEGREE OF CONCENTRATION OF THE URINE

Abscissae, concentration of creatinine in urine/concentration in serum Ordinates, urine inorganic SO<sub>4</sub> milligrams per cent.

The estimated volume of filtrate can be obtained by multiplying the creatinine concentration ratio by the urine volume. In these experiments, it varied from 109–194 cc per minute, and was above 150 in six determinations. These are within the range found by Rehberg, but tend



to be higher than the 100–150 cc per minute usually found under normal conditions

Under special conditions designed to insure maximum kidney function, a relationship between blood concentration and rate of excretion similar to that found for sulphate has been shown in man for urea by Ambard (1931), McLean (1915), Addis (1922) and others and for creatinine by Cope (1931). Such a linear relation is in accord with glomerular filtration of these substances, but is not evidence of absence of back diffusion—for if there were no back diffusion the rate of excretion of each at a given blood level should be the same, while in fact a higher rate is constantly found for creatinine than for urea (Cope (1931), MacKay and Cockrill (1930)). At high blood sulphate levels, the concentration index of sulphate approaches closely that of creatinine, although never quite equalling it. *At normal serum sulphate concentrations, however, the conditions are quite different*

In Table IV are presented the concentration ratio of the urine determined by the quotient (concentration of creatinine in urine/average concentration creatinine in serum during period of urine collection), serum and urine concentrations of urea nitrogen and of inorganic sulphate in normal individuals with various degrees of diuresis. None of the subjects received sulphate. Variations in urine volume were procured by withholding fluid for 12 hours and by ingestion of water. For the greatest urine volumes a liter of water was taken during fifteen minutes, followed by 500 cc every half hour for three or four hours. The estimated volume of filtrate per minute can be obtained from the table by multiplying the creatinine ratio by the urine volume. It varied from 99 to 226 cc per minute. In the 44 determinations, it was below 100 cc once, between 100 and 150 cc twenty-eight times, between 150 and 200 cc eleven times, and above 200 cc four times. This agrees with the findings of Rehberg and of Cope. The data indicate that sulphate is not only concentrated less than creatinine, but with five exceptions, even less than urea. Three of the five instances in which sulphate was more concentrated than urea followed the intramuscular injection of pituitary extract. Neglecting any possible reabsorption or back diffusion of creatinine, the proportion of the filtered sulphate and urea which is excreted can be determined by the formula given by Holten and Rehberg  $E\% = U/Pc \cdot 100$  where  $U$  = urine concentration,  $P$  plasma concentration and  $c$  the concentration ratio of the urine estimated by creatinine. Such calculation shows an excretion per cent for sulphate from 12.9 to 70, average 25.8, and for urea 19.2 to 71.2, average 44.7. The sulphate excreted was less than 30 per cent of that in the calculated filtrate in 34 of 44 observations, between 30 and 50 per cent in 7, and greater than 50 per cent in 3. The urea excreted was below 30 per cent of that in the calculated filtrate in only 4 instances. The increase in excretion percentage of urea with diuresis

TABLE IV

*Concentration ratios of creatinine inorganic sulphate and urea in normal persons*

Name	Urine volume	Creati- nine ratio	Serum SO <sub>4</sub>	Urine SO <sub>4</sub>	SO <sub>4</sub> ratio	Serum urea nitrogen	Urine urea nitrogen	Urea ratio
	<i>cc. per minute</i>		<i>mgm. per 100 cc.</i>	<i>mgm. per 100 cc.</i>		<i>mgm. per 100 cc.</i>	<i>mgm. per 100 cc.</i>	
W	44	286	3.8	322	85.0	13.8	1332	96.5
Buch	.55	221	4.6	125	27.2	8.8	632	71.7
JMH	58	260	3.7	192	52.0	12.2	782	64.1
JMH	64	235	4.5	130	29.0	19.0	1120	59.0
JMH	66	172	4.2	149	35.5	11.5	380	33.0*
JMH	67	191	4.5	145	32.2			
Buch	70	199	4.6	114	24.8	8.8	605	68.7
JMH	76	161	4.7	150	31.9	11.7	683	58.4
Buch	83	119	3.9	87	22.3			
JMH	.87	144	4.5	136	30.2			
Buch	1.16	112	3.9	64	16.4			
Tuck.	1.18	159	4.4	91	20.7	14.1	679	48.1
JMH	1.23	130	3.8	114	30.0	13.9	655	47.1
B	1.26	128	3.6	266	73.9	11.4	470	41.2*
Vict	1.32	85	4.9	156	32.0	13.6	584	43.0
JMH	1.33	111	3.8	97	25.5	13.9	588	42.3
Buch	1.41	101	3.9	82	21.0			
P	1.61	68	4.3	192	44.6	9.7	195	20.0*
JMH	1.77	90	4.5	70	15.5	15.5	574	37.0
Buch	1.79	66	4.6	66	14.3	10.7	378	35.3
L	1.91	66	5.1	141	27.6	10.0	231	23.1
JMH	2.10	78	3.8	59	15.5	13.9	452	32.5
K.	2.21	61	2.9	65	22.4	10.9	335	30.7
Buch	2.52	50	4.6	48	10.4	11.0	292	26.6
T	2.59	54	4.0	70	17.5	11.6	314	27.0
Bailey	2.70	44	6.2	54	8.7	15.5	366	23.6
Buch	2.73	58	4.6	38	8.3	10.7	272	25.4
JMH	3.71	48	4.5	39	8.7	15.2	326	21.4
Lutz	3.80	34	4.9	32	6.5	16.9	259	15.3
Buch	5.07	26	4.6	16	3.5	10.3	153	14.8
Bailey	5.25	23	5.3	23	4.3	14.5	158	10.9
B	5.43	36	3.6	91	25.3	11.4	214	18.8
L	5.68	28	5.1	59	11.6	9.5	126	13.3
Lutz	6.50	20	4.9	29	5.9	16.0	149	9.3
JMH	7.08	21	4.0	22	5.5	13.2	149	11.3
JMH	7.15	17	4.0	24	6.0	13.2	128	9.7
C	8.30	26	4.3	69	16.0	8.5	142	16.7
B	11.17	16	5.1	20	4.0	9.6	65	6.8
JMH	11.30	12	4.2	13	3.1	11.5	92	8.0
Bailey	16.60	8	5.3	9	1.7	15.2	86	5.7
B	18.80	12	5.1	20	4.0	9.4	47	5.0
P	19.40	10	4.3	7	1.6	9.7	61	6.3
Buch	24.50	9	4.7	6	1.3	10.0	44	4.4
Buch	26.60	8	4.7	5	1.1	9.6	37	3.9

\* After intramuscular injection of pituitrin

is not shown for sulphate. The calculated concentration of sulphate in the reabsorbed fluid is always less than the blood concentration. After intravenous injection of sulphate (Table III), the excretion percentage is somewhat higher than at normal blood sulphate levels, reaching a maximum of 92 per cent, but in spite of this the actual amount of sulphate diffusing back from tubule lumen is also greater. The calculated concentration of sulphate in the fluid reabsorbed after sulphate injection is likewise always less than the blood concentration, and the concentration index for sulphate remains within the normal range. A similar limitation of concentrating power for urea was found by Drury (1923) after injection of urea in rabbits. After injection of phosphate its concentration index also approaches that of creatinine, although at normal plasma concentrations it is considerably lower, and according to Havard and Reay (1926) may even fall below one during copious diuresis. We have never encountered urine with a lower sulphate content than blood.

Above a urine volume of about 2 cc per minute the excretion of sulphate is apparently independent of urine volume, the concentration in the urine showing a linear relation to the concentration index of the urine as determined by creatinine (Figure 2). This confirms Poulsson's findings, but if regarded as evidence of lack of back diffusion of sulphate, leads to calculated blood sulphate contents from 12 to 40 per cent of those found by analysis. With low urine volumes and high concentrations, the excretion of sulphate diminishes. This is similar to the findings for urea of Austin, Stillman and Van Slyke (1921), and Moller, McIntosh and Van Slyke (1928). Indeed, when the quantity urine sulphate/blood sulphate  $\times$  urine volume, representing the number of cubic centimeters of blood whose sulphate content is contained in one minute's urine, is plotted against urine volume, curves of the same type are obtained as those plotted by Moller, McIntosh and Van Slyke for urea excretion (Figure 3).

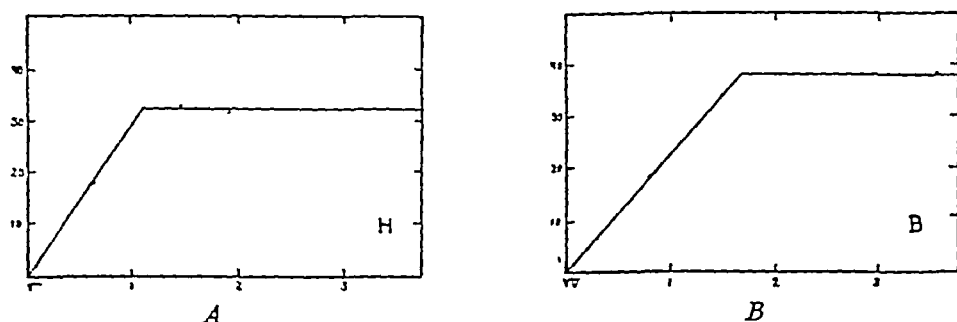


FIG 3, A and B SULPHATE EXCRETION CURVES FROM TWO NORMAL SUBJECTS PLOTTED AS MÖLLER, MCINTOSH AND VAN SLYKE'S CURVES FOR UREA EXCRETION

Abscissae, square root of urine volume (cc per minute). Ordinates, cubic centimeters of blood cleared of inorganic sulphate by one minute's excretion ( $U \cdot V/B$ , where  $U$  = concentration inorganic sulphate in urine,  $B$  = concentration in serum, and  $V$  = cc. urine per minute).

There is more scattering of the points in these charts than in similar charts for urea excretion. This is probably attributable to the fact that our blood sulphate analyses are not as accurate as the analysis for urea nitrogen. The "augmentation limit," or urine volume above which increased diuresis does not lead to increased excretion, is apparently between 1 and 2 cc per minute, which agrees with the range 1.67 to 2.55 found for urea excretion by Möller, McIntosh and Van Slyke.

The explanation offered by Holten and Rehberg for the relationship of urea excretion and urine volume, namely, that extent of back diffusion is not strictly proportional to volume and concentration of the urine, but is relatively greater with diuresis, may also be offered for sulphate.

The excretion of sulphate in nephritis appears to be of the same nature as in the normal (Table V). When blood sulphates are normal, the concentration ratio of sulphate and creatinine have the usual relationship to each other while when blood sulphates are elevated the sulphate ratio approaches that of creatinine as it does after injection of sulphate in a normal person. We confirm Wakefield, Power and Keith's (1931) observation that serum inorganic sulphates are often elevated before urea nitrogen. This is consistent with the view expressed here that back diffusion of sulphate is usually greater than that of urea. On the other hand, in some patients with uremia and marked elevation of both blood sulphate and urea nitrogen, the concentration ratio and excretion of sulphate may be greater than of urea nitrogen.

These studies do not prove the mechanism of excretion of inorganic sulphates. In order to prove that sulphates are eliminated by filtration their presence in glomerular fluid in the same concentration as in blood plasma would have to be established. To prove back diffusion, it would have to be shown that they are less concentrated in the urine than some substance already shown to be eliminated only by glomerular filtration. Evidence of tubular elimination could only be obtained by showing that they were present in urine in greater amount than could possibly be accounted for by glomerular filtration. Convincing evidence of glomerular filtration has been presented by Richards (1929) and his associates, and proof of reabsorption of sugar and chlorides by Wearn and Richards (1924). If belief in glomerular filtration be accepted, creatinine after its ingestion and sulphate can be accepted as present in the filtrate. The increased elimination of creatinine with decreases in plasma proteins (N<sub>1</sub> and Rehberg, 1931), the close agreement in concentration ratios of creatinine and sugar in the phloridzinized animal (Poulsen, 1930b), and the fact that the volume of glomerular filtrate necessary to account for the elimination of creatinine by filtration alone is not inconsistent with the area of filtering surface (Vimtrup, 1928) and observed rates of passage of fluid through capillary walls (Landis, 1927), makes it unnecessary to fall back on tubular secretion to account for the elimination of creatinine.

TABLE V  
Concentration ratios of creatinine, inorganic sulphate, and urea in nephritic patients

Name	Urine volume cc per minute	Creatinine ratio	Serum SO <sub>4</sub> mgm per 100 cc	Urine SO <sub>4</sub> mgm per 100 cc	SO <sub>4</sub> ratio	Serum urea nitrogen mgm per 100 cc	Urine urea nitrogen mgm per 100 cc	Urea ratio	Diagnosis
DeG	1.35	38.8	5.9	55	9.3	14.9	315	21.1	Nephrosclerosis
Cosby	1.75	15.5	4.6	26	5.6	12.8	112	8.8	Malignant hypertension
Durben	1.95	23.5	5.0	44	8.8	12.4	192	15.5	Chronic nephritis
Ryan	2.92	16.0	5.4	36	6.7	11.3	135	11.9	Subacute nephritis
Ryan	4.70	18.7	4.6	21	4.6	7.5	107	14.3	
Lewis	5.25	40.0	5.5	15	2.7	6.2	103	16.6	Subacute nephritis
Stephens	6.33	15.5	5.6	13	2.3	14.2	78	5.5	Nephrosclerosis
Bonath	7.70	16.4	6.2	13	2.1	9.1	85	9.3	Acute nephritis
Christofson	8.75	9.8	4.7	15	3.2	11.6	66	5.7	Nephrosclerosis
James	10.00	12.0	3.7	16	4.3	8.1	87	10.7	Nephrosclerosis
Gardner	0.11	3.9	91.4	263	2.8	163.0	259	1.6	Nephrosclerosis, uremia
Verhoben	0.43	7.5	31.5	69	2.2	88.7	343	3.9	Chronic nephritis, uremia
Johnson	0.48	83.0	7.8	260	33.3	17.4	705	40.5	Nephrosclerosis
Few	0.49	7.7	55.1	216	3.9	131.5	437	3.3	Chronic nephritis, uremia
Lewis	0.69	9.6	17.0	103	6.1	60.0	296	4.9	Chronic nephritis
Kish	1.01	45.5	8.8	141	16.0	14.0	221	15.8	Chronic nephritis
Hraster	1.08	7.5	11.2	54	4.8	52.4	193	3.7	Chronic nephritis
Conroy	1.35	28.3	8.3	66	7.9	34.9	378	10.8	Chronic nephritis
Stankos	1.69	29.4	8.3	92	11.1	12.1	152	12.6	Amyloidosis
Zuparcsek	2.05	7.6	28.7	162	5.6	83.3	410	4.9	Nephrosclerosis
Klienfeldt	2.21	2.2	43.7	65	1.5	137.0	212	1.6	Pyelonephritis
Smith	2.46	13.2	8.2	25	3.0	18.0	187	10.4	Nephrosclerosis
Stankos	2.76	23.8	8.3	77	9.3	11.0	125	11.4	Amyloidosis
Anzo	3.03	23.0	7.0	44	6.3	19.1	185	9.7	Chronic nephritis
Conroy	4.50	12.4	8.7	87	10.0	12.5			Chronic nephritis

If inorganic sulphate is believed to be present in the glomerular filtrate, rates of excretion less than for creatinine seem most readily accounted for by diffusion of some sulphate ion from the lumen of the tubules into tubule cells during the process of concentration of the urine by the active reabsorption of water. The elimination of such substances as urea and sulphate depends in part on the capacity of the tubule cells to resist this back diffusion—to preserve a high concentration on the lumen side and a low one on the capillary side. Our results indicate that this power is usually greater for urea than for sulphate.

#### CONCLUSION

1 Inorganic sulphates are less concentrated by the human kidney than creatinine, and usually less concentrated than urea. In accordance with a belief in filtration and reabsorption, this is regarded as evidence that some sulphate diffuses back through tubule cells.

2 After intravenous injection of sulphate, the concentration ratio approaches that of creatinine.

3 In nephritis with elevated serum sulphate, the excretion resembles that in a normal individual after injection of sulphate.

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# ON THE MECHANISM OF NEPHROTIC EDEMA

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## INTRODUCTION

The study of factors responsible for the development of edema in various disease conditions has led to the accumulation of much valuable data, but a fundamental understanding of the physicochemical processes remains to a large extent obscure. Furthermore, a correlation of the mechanisms that are understood has perhaps been delayed by the tendency of various groups of investigators to overemphasize a single mechanism. The confusion is probably greatest in that type of edema seen most frequently in chronic nephritis or nephrosis<sup>1</sup> and also in that known as "nutritional edema."

The edema of chronic nephritis has been explained by one group of investigators upon the hypothesis that renal or tissue defects interfere with the elimination of certain electrolytes and by another group upon the theory that the fundamental disturbance is a decrease in the protein content of the blood serum. The chief exponents of the former school of thought are Widal (1), who pointed out the apparent inability of the kidney to excrete the chloride ion, and Magnus Levy (2, 3), Leon Blum and his pupils (4) and Falta (5), who have emphasized the apparent inability of the kidney to excrete sodium in the presence of nephrotic edema. In 1896 Starling (6) opened the way for the alternative concept when he pointed out that if the osmotic pressure of the blood serum in the capillaries fell below the hydrostatic pressure, fluid would be forced into the tissue spaces and consequently could not be absorbed. This idea was first applied to the mechanism of edema in renal disease by Epstein (7) in his studies of nephrosis. In the past decade, as a result of extensive studies, Peters and his co-workers (8) have extended this concept and have shown the striking parallelism between the protein content of the serum and a tendency to the development of edema. They have shown that this relationship holds not only for nephrotic but also for nutritional edema where there is no reason to assume the presence of

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<sup>1</sup> In this paper the expression "nephrotic edema" will be applied to that type of edema found in patients suffering either from true lipid nephrosis or the so-called nephrotic form of chronic glomerular nephritis.

kidney damage Their findings are in accord with the careful observations of Govaerts (9) and of Schade (10) and also receive confirmation in the recent work of Leiter (11), Barker and Kirk (12), Weech and Ling (13), Paul Meyer (14) and many others

While the general parallelism between the presence of edema and decrease in the concentration of protein in the blood with its accompanying abnormality in the ratio, albumin/globulin, is striking, certain factors disturbing to the assumption of a simple causal relationship have been encountered In 1923, the writers (15) found that edema of the nephrotic type might disappear completely without change in the concentration of proteins in the blood Since that time Van Slyke and associates (16) and others have observed the same phenomenon Paul Meyer (14) has gone a step further and found that edema of this type may disappear while the "colloidal" osmotic pressure of the serum actually diminishes significantly, whereas a rise would naturally be anticipated according to the osmotic hypothesis Unless, therefore, there is a striking drop in the hydrostatic pressure in the venous limbs of the capillaries in these cases, which does not seem likely, it is difficult to explain the process of edema on the basis of low serum protein alone Other facts difficult to explain on this purely osmotic basis are the experiments of Magnus-Levy and of L. Blum, which suggest that there is specific retention of sodium but not of potassium in certain edematous individuals From a purely osmotic or hydrostatic viewpoint, one would anticipate approximately equal retention of any monovalent ion with water This specific sodium retention in nephritis has been frequently attributed to local kidney damage On the other hand in nutritional edema it is difficult to conceive of the presence of local kidney changes and yet in this condition disturbances of water and electrolyte excretion similar to those present in nephrosis are encountered

In view of this contradictory state of our knowledge, it has seemed worth while to study in prolonged and carefully controlled balance experiments, the response to the ingestion of various electrolytes in a patient with nephrotic edema and to contrast the behavior of this individual with the normal under similar circumstances.

#### METHODS

The methods employed for the determination of chloride, total base (except that in the stool, food, and urine when the calcium was higher than normal, the calcium was removed and sodium, potassium and magnesium determined together), carbon dioxide capacity, nonprotein nitrogen, phosphates, total nitrogen and hematocrit are described in an earlier paper (24) The calcium content of serum, urine and stool was determined by the method of Clark and Collip (25) Potassium was determined by the method of Tisdall and Kramer (26) with the exception that the serum was ashed and that in the urine determinations 1 cc was ashed, made up to a volume of 10 cc and 2 cc aliquots

analyzed Organic acids were determined by the method of Van Slyke and Palmer (27) Ketones were determined by Van Slyke's method (28) and creatinine by Folin's method (29) For oxygen capacity the method of Van Slyke and Neill (30) was employed Sugar was determined by the method of Folin and Wu (31) Inorganic sulfates were estimated by Fiske's method (32) with the following alteration in the removal of phosphate the magnesium carbonate was added before instead of after making up to volume 15 cc to 20 cc of filtrate were used, which after precipitation were centrifuged for 20 minutes (2000 r p m) instead of being filtered The precipitate was washed by adding one cc of 95 per cent acetone and mixing with a glass rod which was washed with 3 cc. of acetone and centrifuged for 20 minutes This was repeated twice, 5 cc. of water were added to the precipitate which was heated in a water bath until all odor of acetone was gone Titratable acids were determined as follows a standard of 10 cc. of M/5 phosphate buffer mixture at pH 7.4 plus three drops of 0.5 per cent aqueous neutral red was used 10 cc of urine in a 200 cc volumetric flask with three drops of 0.5 per cent neutral red were titrated with N/10 sodium hydroxide and reported as milliequivalents of acid per twenty four hours The base bound to phosphate was calculated according to Gamble, Ross and Tisdall (33), and base bound to protein according to Van Slyke, Wu and McLean (34)

## PART I THE STUDY OF A PATIENT WITH NEPHROTIC EDEMA

### *Details of the experiment*

*A G number 295241* The patient was an American born Jewish boy of 21 whose family and past history were entirely negative except for the fact that his mother has hypertension Without any preceding infection, three months before admission the patient complained of mild generalized pruritus for a week and this was followed by the gradual development of massive edema of the face, abdomen genitalia and legs, for which the patient sought relief after eleven weeks at home. Physical examination including the eye grounds was entirely negative except for the edema Blood pressure was 122/88 The urine always showed a heavy trace of albumin, specific gravity varied between 1.012 and 1.030 The sediment contained granular and hyalin casts most of the time and very occasionally a few red blood cells On admission his phthal ein excretion was 40 per cent Routine blood counts and hemoglobin were normal The blood Wassermann reaction was negative. During his stay of four and one half months in the hospital, the blood serum protein varied between 3.2 and 3.7 per cent blood urea between 0.71 and 0.23 grams per liter cholesterol between 440 and 233 mgm per 100 cc., blood calcium between 8.8 and 9.5 mgm per 100 cc. The blood phosphorus was 4.8 mgm per 100 cc. The basal metabolism varied between -26 and -7 per cent The blood chloride varied between 100 and 119 m eq per liter depending more or less on the type of therapy being employed While in the hospital he was given a diet high in protein and containing less than 2.0 grams of NaCl a day He received many types of therapy, most of which were totally ineffectual in bringing about a diuresis Thyroid extract was gradually increased to 1.80 grams daily without symptoms Coincidentally with this thyroid administration and the ingestion of 12.0 grams of  $\text{NH}_4\text{Cl}$  daily the patient gradually lost 42 pounds This diuresis was not accompanied by any significant change in the concentration of protein in the blood serum On admission the serum protein concentration was 3.5 per cent and after diuresis it was 3.7 per cent

*Second admission:* Four weeks after his discharge from the hospital, the patient was readmitted to the hospital, having gained 32 pounds in spite of the daily ingestion of 80 grams of  $\text{NH}_4\text{Cl}$  on alternate weeks. Physical examination was again essentially negative, except for massive edema. His urinary findings were unchanged and his blood count showed no significant abnormality. The serum protein concentration was 3.5 per cent, the blood cholesterol was 356 mgm per cent, his blood chloride concentration was 110 m eq per liter (probably effect of daily ingestion of  $\text{NH}_4\text{Cl}$ ) and the blood urea was 0.41 gram per liter. His basal metabolic rate was -27 per cent.

On admission, all medication was stopped, the patient was given a high protein diet containing less than 20 grams of  $\text{NaCl}$  a day. He remained in bed during the entire period of experimental observation. On September 21, ten days after admission, a still more carefully controlled regime was instituted. The patient was placed on a standard diet of 80 grams of protein and enough carbohydrate and fat were added to make a daily intake of 2000 calories and to yield a neutral ash. The mineral content of the diet was calculated to contain the following amount daily:  $\text{K} = 76.5$  m eq,  $\text{Na} = 29.8$  m eq,  $\text{Ca} = 20.5$  m eq,  $\text{Mg} = 19.5$  m eq,  $\text{Cl} = 19.6$  m eq and  $\text{P} = 65.0$  m eq calculated at pH 7.0. The patient ate identical meals every day of the period of observation and was given 1000 cc of fluid in addition to the water of the food. The patient ate his entire quota of food every day and there was never any diarrhea. The observations were carried on in the autumn and no visible sweating occurred.

The studies on this patient were limited chiefly to the urinary response to the ingestion of the chlorides of potassium, sodium, ammonium and calcium. Observations were made on the blood from time to time, the samples being taken without the use of the tourniquet and delivered under oil. When calcium chloride was given the loss of these two ions in the feces was determined. All analyses were made in duplicate. The pH of the urine, titratable acid and  $\text{NH}_3$  were determined daily. Total fixed base, potassium and chlorine were determined for periods varying from two to four days, as may be seen in Table 1. In the last two periods calcium was determined in the urine. In the other periods Na, Mg and Ca were estimated together by the difference between total fixed base and potassium. This seemed a justifiable procedure, as the amounts of these three ions formed, in most instances, only a small fraction of the total base excreted.

## RESULTS

In the foreperiod which consisted of twelve days divided into units of three days each there were only minor fluctuations in the excretion of total inorganic base,  $\text{K}$ ,  $\text{Na} + \text{Mg} + \text{Ca}$  as determined by difference,  $\text{Cl}$ , and  $\text{NH}_3$ . These constant rates of excretion obtained particularly after the third day, as may be seen in Figure 1 and Table 1. There were distinct fluctuations in the urine pH and titratable acid indicative of irregularity in phosphate excretion and possibly of varying degrees of proteinuria. There were also fluctuations in the excretion of nitrogen.

*Effect of the ingestion of KCl:* During the two days of period V the patient was given 260 m eq of  $\text{KCl}$  with marked changes in the composition of the urine. There was a drop in the excretion of  $\text{NH}_3$ , the titratable acid decreased strikingly with a coincident increase in the pH. These

changes appear to be explained by the interesting fact that 66 per cent of the ingested potassium was excreted in these days while only 35 per cent of the extra Cl was eliminated. On the first day of period VI, 37 m eq of KCl were ingested and then KCl administration was stopped. In this period (see Table 1 and Figure 1) the urine pH went down, the titratable acid increased and there was a sustained increase in the  $\text{NH}_4$  excretion accompanied perhaps by a slight increase in the base fraction  $\text{Na} + \text{Mg} + \text{Ca}$ . This behavior of the urine may find its explanation

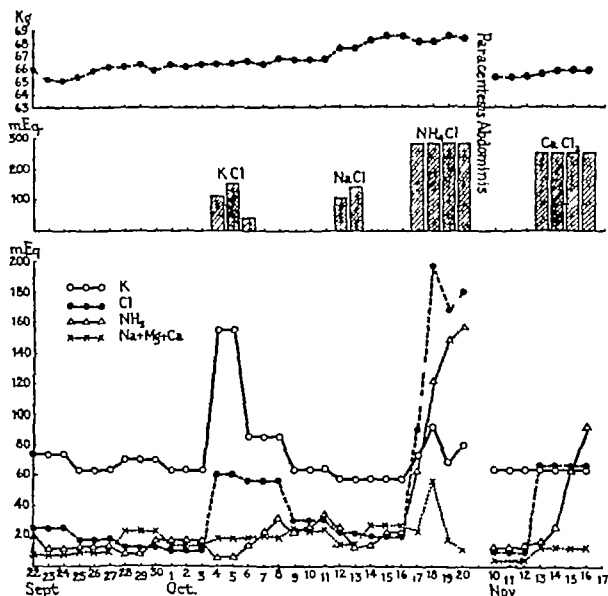


FIG 1 URINARY RESPONSE OF THE NEPHROTIC PATIENT TO THE INGESTION OF VARIOUS ELECTROLYTES

in the fact that during these three days only 17 per cent of the total K added to the diet was excreted whereas 43 per cent of the chlorine passed through the kidneys. In other words, the reaction resembled that of the body to the ingestion of HCl or an "acid salt." No diuresis resulted in this patient from this short period of KCl administration.

*Effect of the ingestion of NaCl* During the two days of period VIII the patient was given 246 m eq of NaCl. This was quantitatively retained by the body as is strikingly shown in Figure 1. There was no apparent effect on the rate of excretion of  $\text{NH}_4$ , titratable acid,  $\text{Na} + \text{Mg}$

TABLE 1  
*Urine study in the patient with chronic nephritis*

Period	Date	Body weight kgm	Urine vol- ume cc	Urine pH	Titrat- able acid m eq	Urine NH <sub>4</sub> m eq	Urine total fixed base m eq	Urine K m eq	Urine Na+Mg+Ca m eq	Urine Cl m eq	Urine N grams	Remarks
I	September 22	66.0	740	5.8	31.7	20.4	82.3*	74.0*	8.3*	23.9*		
	September 23	65.4	615	5.9	25.5	13.0	82.3	74.0	8.3	23.9	9.8	
	September 24	65.3	560	5.9	30.4	11.4	82.3	74.0	8.3	23.9	9.6	
II	September 25	65.5	650	5.8	27.5	13.9	74.0*	62.7*	11.3*	15.2*	10.0	
	September 26	66.0	535	5.8	25.2	14.7	74.0	62.7	11.3	15.2	8.4	
	September 27	66.2	1085	6.1	23.3	14.2	74.0	62.7	11.3	15.2	10.4	
III	September 28	66.2	980	6.3	16.4	10.0	90.4*	71.0*	19.4*	11.9*	9.5	
	September 29	66.9	1225	6.5	14.0	11.4	90.4	71.0	19.4	11.9	10.8	
	September 30	65.9	1000	6.2	21.8	14.7	90.4	71.0	19.4	11.9	10.1	
IV	October 1	66.3	895	6.3	17.6	13.6	74.8*	62.0*	12.8*	10.0*	10.0	
	October 2	66.2	720	6.1	19.4	14.8	74.8	62.0	12.8	10.0	9.3	
	October 3	66.3	890	6.2	22.5	14.2	74.8	62.0	12.8	10.0	10.1	
V	October 4	66.4	555	6.6	3.6	5.2	172.5*	154.5*	18.0*	60.5*	8.7	Received 111 m eq extra KCl
	October 5	66.5	915	7.45	-1.4	5.7	172.5	154.5	18.0	60.5	11.0	Received 149 m eq extra KCl
VI	October 6	66.8	720	6.0	23.6	14.3	104.0*	85.2*	18.8*	56.3*	10.5	Received 37 m eq extra KCl
	October 7	66.6	635	5.45	33.2	20.9	104.0	85.2	18.8	56.3	10.6	
	October 8	67.0	760	5.6	33.4	32.4	104.0	85.2	18.8	56.3	11.0	
VII	October 9	66.8	1280	6.1	25.0	22.9	87.5*	63.8*	23.7*	30.2*	11.3	
	October 10	66.8	1015	5.9	30.6	26.5	87.5	63.8	23.7	30.2	11.5	
	October 11	66.8	970	5.9	28.4	33.3	87.5	63.8	23.7	30.2	11.8	

TABLE 1 (continued)

Period	Date	Body weight gms.	Urine vol cc.	Urine pH	Titrat able acid	Urine NH <sub>3</sub>	Urine total fixed base	Urine K	Urine Na+Mg+Ca	Urine Cl	Urine N	Remarks
VIII	October 12	67.8	505	5.9	26.2	23.4	73.8*	58.4*	15.4*	22.4*	9.9	Received 106 m.eq extra NaCl
	October 13	67.8	495	6.1	20.1	14.2	73.8	58.4*	15.4	22.4*	7.1	Received 140 m.eq extra NaCl
IX	October 14	68.4	540	6.1	21.6	15.1	84.7*	57.7*	27.0*	19.8*	10.7	
	October 15	68.6	855	5.9	22.6	21.2	84.7	57.7	27.0	19.8	11.0	
	October 16	68.6	845	5.9	28.9	20.9	84.7	57.7	27.0	19.8	10.2	
X	October 17	68.4	945	5.4	36.4	62.4	95.8	73.6	22.2	90.4	13.8	Received 281 m.eq extra NH <sub>4</sub> Cl
	October 18	68.3	1430	5.0	44.5	122.4	148.8	92.6	56.2	196.3	17.4	Received 281 m.eq extra NH <sub>4</sub> Cl
	October 19	68.8	1060	5.7	33.0	148.5	87.5	69.3	18.2	168.0	15.2	Received 281 m.eq extra NH <sub>4</sub> Cl
	October 20	68.6	1040	5.9	33.6	157.5	91.4	79.5	11.9	180.0	15.2	Received 281 m.eq extra NH <sub>4</sub> Cl
												Paracentesis abdominis performed
XI	November 10	65.6	890	5.5	28.4	12.2	68.3*	63.5*	4.8*	10.4*	10.2*	
	November 11	65.6	860	5.6	27.7	11.9	68.3	63.5	4.8	10.4	10.2	
	November 12	65.4	755	5.5	29.0	13.0	68.3	63.5	4.8	10.4	10.2	
XII	November 13	65.8	700	5.5	16.9	16.8	78.1*	63.7*	14.4*	66.3*	12.0*	Received 271 m.eq extra CaCl <sub>2</sub>
	November 14	66.0	710	5.4	17.9	36.2	78.1	63.7	14.4	66.3	12.0	Received 271 m.eq extra CaCl <sub>2</sub>
	November 15	66.0	740	5.4	22.2	63.7	78.1	63.7	14.4	66.3	12.0	Received 271 m.eq extra CaCl <sub>2</sub>
	November 16	66.0	900	5.4	32.1	92.0	78.1	63.7	14.4	66.3	12.0	Received 271 m.eq extra CaCl <sub>2</sub>

\* Average daily output for the period



+ Ca, or on the excretion of Cl. The K excretion in this and the following period seemed to be slightly decreased. The pH of the urine was not influenced. The patient gained 1.8 kgm as a result of the NaCl ingestion. The difference between this edematous patient's physiological response to the ingestion of the K and Na salts of the same acid is indeed impressive. In the case of KCl, the cation and anion were both eliminated, the former more rapidly than the latter. This phenomenon may perhaps be explained by the much greater relative rate of "reabsorption" of the chloride ion in the tubules of the kidney. Differences in ionic mobilities will not explain the differential rate of excretions of the K and Cl ions, as they are almost identical. Chloride given with the cation Na was not excreted at all.

*Effect of the ingestion of  $\text{NH}_4\text{Cl}$*  During the four days of period X the patient was given 281 m eq of  $\text{NH}_4\text{Cl}$  daily. The excretion of  $\text{NH}_3$  and of Cl rose enormously. On the second day of administration of  $\text{NH}_4\text{Cl}$ , the excretion of potassium increased about 25 m eq above the average for the control periods and the combined base fraction  $\text{Na} + \text{Mg} + \text{Ca}$  increased about 40 m eq and then fell in spite of the well sustained excretion of  $\text{NH}_3$  and Cl. It is of interest to note in this patient that no diuresis resulted from the administration of large doses of  $\text{NH}_4\text{Cl}$  and that the loss of fixed base from the body was minimal in spite of a marked lowering of the bicarbonate in the blood (to 20.0 m eq per liter).

*Effect of the ingestion of  $\text{CaCl}_2$*  In period XII of the observations, the patient received 271 m eq of  $\text{CaCl}_2$  daily for four days. As in the case of  $\text{NH}_4\text{Cl}$  administration, there was an increase in  $\text{NH}_3$  and titratable acid excretion and a definite increase in Cl output by the kidneys but the changes were much less marked, as is seen in Table 1 and Figure 1. After four days, the concentration of  $\text{HCO}_3'$  in the blood serum fell from 27.5 to 13.5 m eq per liter without diuresis or a significant loss of fixed base from the body.

It is of interest that stools collected for two days during the period of  $\text{CaCl}_2$  feeding contained 209 m eq of Ca daily, whereas the total calcium excreted daily by the kidneys in this period was only 1.8 m eq a day. In the control foreperiod XI the daily urinary Ca excretion was only 0.2 m eq, a very low value indeed.

*Effects of various electrolytes on the blood serum* Chloride administered as KCl,  $\text{NH}_4\text{Cl}$  or  $\text{CaCl}_2$  caused an increase in Cl concentration in the serum and the two latter salts when given in massive doses over some days caused a significant fall in bicarbonate. In spite of the acidosis induced by  $\text{NH}_4\text{Cl}$  and  $\text{CaCl}_2$  no diuresis resulted. This might be attributed to the low concentration of base present in the serum as seen in Table 2. Albright and Bauer (17), in a detailed study of a patient with chronic nephrotic edema, concluded that diuresis was brought about by raising the base concentration above a critical level. Peters expressed

TABLE 2

*Blood serum studies on the patient with chronic nephritis*

Date	Total base	Potassium	Bicarbonate	Chloride	Serum protein	Remarks
	m. eq per liter	m. eq per liter	m. eq per liter	m. eq per liter	per cent	
10-5	146.5	5.5	26.7	107.0	3.4	After receiving 111 m. eq KCl on October 4, 1931
10-19	148.0	4.4	20.0	109.2	4.0	After receiving 281 m. eq $\text{NH}_4\text{Cl}$ daily for 3 days
11-12	145.9		27.5	101.0	3.0	At end of control period XI
11-17	145.0		13.5	116.6	3.4	After receiving 271 m. eq $\text{CaCl}_2$ for 4 days

the same opinion. Osman (18, 19) has made similar observations but an interpretation of his results is confused by the fact that sodium and potassium bicarbonates were administered simultaneously and by the fact that no serum base measurements were made. While the low concentration of base in the serum may be a factor in the inability of patients to excrete sodium chloride, this alone cannot be considered the determining factor, as the writers have observed a diabetic patient, whose serum base was reduced to 146.5 m. eq per liter, become markedly dehydrated while excreting over 200 m. eq of sodium in the course of twenty-four hours. In another diabetic 117.6 m. eq of sodium and magnesium determined together were excreted in twenty-four hours, while the concentration of inorganic base in the blood serum was 146.8 m. eq per liter. The serum protein concentration was normal in both of these patients. It is possible that when the inorganic base level of the blood serum is markedly reduced and when the concentration of protein in the serum is simultaneously found to be low, difficulties in water and sodium excretion exist. This statement offers no explanation but would seem to indicate that the problem deserving further study is that of the factors which determine the conditions under which the sodium ion may be excreted by the kidney.

## PART 2 THE STUDY OF ELECTROLYTE BALANCES IN A NORMAL ADULT

There are numerous studies on the effects of various electrolytes upon human subjects in health and in disease. Because of the tediousness of prolonged balance studies and the technical difficulties encountered in making complete and simultaneous analyses of food, urine, stools and blood most of the reports are inconclusive. For this reason and for the purpose of comparison of the behavior of the normal with the diseased human subject under various conditions we have made detailed observa-

tions on several normal young men over long periods of time. The results of one of these studies are presented below. Observations in this case were made on the response to the ingestion of potassium chloride, sodium chloride and ammonium chloride as in the patient suffering from nephrosis already described.

### *Details of the experiment*

*E F, number 316121* The subject was a healthy unemployed adult white male of 23 whose only illnesses in the past were pneumonia five years before admission and a sore throat three years ago. On physical examination he was well developed and well nourished though lean, but no abnormalities were noted. Routine blood counts and urine examinations were normal. During the course of the experiment, which lasted 45 days, the patient remained in bed except for six hours a day when he was permitted to sit in a chair and to walk a few steps. He was placed on a standard diet immediately after admission and ate identical meals each day throughout the period of observation. There was never any return of food to the kitchen and there was at no time diarrhea. The diet was calculated to contain 85 grams protein and enough carbohydrate and fat to make a total of 2645 calories. The amount of fluid added to the diet was constant. Every five days when fresh foods were obtained, a complete duplicate day's diet was analyzed for fluid content, total inorganic base, K, Ca, Na + Mg by difference, Cl, P and total N. The urine was analyzed daily for titratable acid,  $\text{NH}_3$ , total inorganic base, K, Ca, Na + Mg by difference, Cl, P, inorganic  $\text{SO}_4$ , total N, organic acids and creatinine. pH determinations were also made daily. The stools were collected in five-day periods and at the end of each period an enema of 500 cc. of distilled water was given. The stools were then analyzed for total inorganic base, K, Na + Mg by difference, Cl, P, and total N. Blood studies were made from time to time, the blood being taken without stasis and delivered as usual under oil. All analyses were made in duplicate throughout the experiment.

### RESULTS

*The foreperiod* After the patient had been on the standard regime for a period of seven days, analyses were begun and were made daily for six days more as may be seen in Table 3 and Figure 2. The chief point of interest is the extraordinary constancy of the urinary findings in this normal individual in contrast to the wider fluctuations seen in the nephrotic patient.

*The effect of the ingestion of KCl* After the completion of the foreperiod, the patient was given 201 m eq of KCl on one day and on the subsequent day 134 m eq. It may be seen from Table 3 and Figure 2 that the effects were similar to those observed in the nephrotic patient. On the first day of KCl administration there was an abrupt increase in the pH of the urine and a drop in the titratable acid, with perhaps a slight decrease in the  $\text{NH}_3$  excreted. On the following two days, the pH decreased and the titratable acid rose above the levels of the control period. These changes, as in the case of the nephrotic patient, were related to differences in the rate of excretion of the K and Cl ions. On

Date	Intake									Remarks
	Potas- sium	Sodium and mag- nesium (by differ- ence)	Cal- cium	Total Inor- ganic base	Chlo- ride	Phos- phate	Phos- phate	Total nitro- gen	Cal- ories	
October 30-31	81.8	38.2	23.3	143.3	19.2	1.28	74.8	12.4	2645	
October 31-										
November 1	81.8	38.2	23.3	143.3	19.2	1.28	74.8	12.4	2645	
November 1-2	81.8	38.2	23.3	143.3	19.2	1.28	74.8	12.4	2645	
November 2-3	81.8	38.2	23.3	143.3	19.2	1.28	74.8	12.4	2645	
November 3-4	81.8	38.2	23.3	143.3	19.2	1.28	74.8	12.4	2645	
November 4-5	79.9	42.8	22.8	145.5	19.1	1.18	68.8	12.6	2645	
November 5-6	280.9	42.8	22.8	346.5	220.1	1.18	68.8	12.6	2645	KCl feeding
November 6-7	213.9	42.8	22.8	279.5	153.1	1.18	68.8	12.6	2645	KCl feeding
November 7-8	79.9	42.8	22.8	145.5	19.1	1.18	68.8	12.6	2645	
November 8-9	79.9	42.8	22.8	145.5	19.1	1.18	68.8	12.6	2645	
November 9-1	81.9	41.2	22.6	145.7	18.7	1.16	67.6	12.3	2645	
November 10-	81.9	41.2	22.6	145.7	18.7	1.16	67.6	12.3	2645	
November 11-	81.9	41.2	22.6	145.7	18.7	1.16	67.6	12.3	2645	
November 12-	81.9	41.2	22.6	145.7	18.7	1.16	67.6	12.3	2645	
November 13-	81.9	41.2	22.6	145.7	18.7	1.16	67.6	12.3	2645	
November 14-	275.6	46.1	22.1	343.8	219.6	1.18	68.6	12.3	2645	KCl feeding
November 15-	208.6	46.1	22.1	276.8	152.6	1.18	68.6	12.3	2645	KCl feeding
November 16-	74.6	46.1	22.1	142.8	18.6	1.18	68.6	12.3	2645	
November 17-	74.6	46.1	22.1	142.8	18.6	1.18	68.6	12.3	2645	
November 18-	74.6	46.1	22.1	142.8	18.6	1.18	68.6	12.3	2645	
November 19-	76.0	40.4	22.3	138.7	18.6	1.23	71.4	12.4	2645	
November 20-	76.0	40.4	22.3	138.7	18.6	1.23	71.4	12.4	2645	
November 21-	76.0	40.4	22.3	138.7	18.6	1.23	71.4	12.4	2645	
November 22-	76.0	40.4	22.3	138.7	18.6	1.23	71.4	12.4	2645	
November 23-	76.0	40.4	22.3	138.7	242.6	1.23	71.4	12.4	2645	NH <sub>4</sub> Cl feeding
November 24-	70.7	56.5	23.5	150.7	243.7	1.26	73.2	12.5	2645	NH <sub>4</sub> Cl feeding
November 25-	70.7	56.5	23.5	150.7	243.7	1.26	73.2	12.5	2645	NH <sub>4</sub> Cl feeding
November 26-	70.7	56.5	23.5	150.7	19.7	1.26	73.2	12.5	2645	
November 27-	70.7	56.5	23.5	150.7	19.7	1.26	73.2	12.5	2645	
November 28-	70.7	56.5	23.5	150.7	19.7	1.26	73.2	12.5	2645	
November 29-	77.3	44.2	22.9	144.4	19.5	1.36	79.0	12.4	2645	
November 30-										
December 1	77.3	44.2	22.9	144.4	19.5	1.36	79.0	12.4	2645	
December 1-2	77.3	163.9	22.9	264.1	139.2	1.36	79.0	12.4	2645	NaCl feeding
December 2-3	77.3	163.9	22.9	264.1	139.2	1.36	79.0	12.4	2645	NaCl feeding
December 3-4	77.3	163.9	22.9	264.1	139.2	1.36	79.0	12.4	2645	NaCl feeding
December 4-5	75.0	164.9	23.1	263.0	140.3	1.23	71.4	12.0	2645	NaCl feeding
December 5-6	75.0	164.9	23.1	263.0	140.3	1.23	71.4	12.0	2645	NaCl feeding
December 6-7	75.0	164.9	23.1	263.0	140.3	1.23	71.4	12.0	2645	NaCl feeding
December 7-8	75.0	164.9	23.1	263.0	364.3	1.23	71.4	12.0	2645	NH <sub>4</sub> Cl + NaCl feeding
December 8-9	75.0	164.9	23.1	263.0	364.3	1.23	71.4	12.0	2645	NH <sub>4</sub> Cl + NaCl feeding
December 9-10										

\* Twenty-

† Twenty-



the first day of KCl ingestion, 70.9 per cent of the K ingested was excreted in the urine, whereas only 51 per cent of the Cl was eliminated in that time. During the next twenty four hours, some of the chlorine which had been retained was excreted, so that at the end of 48 hours of KCl administration 78.6 per cent of the ingested K and 74.5 per cent of the Cl were recovered in the urine. In the days following the ingestion of

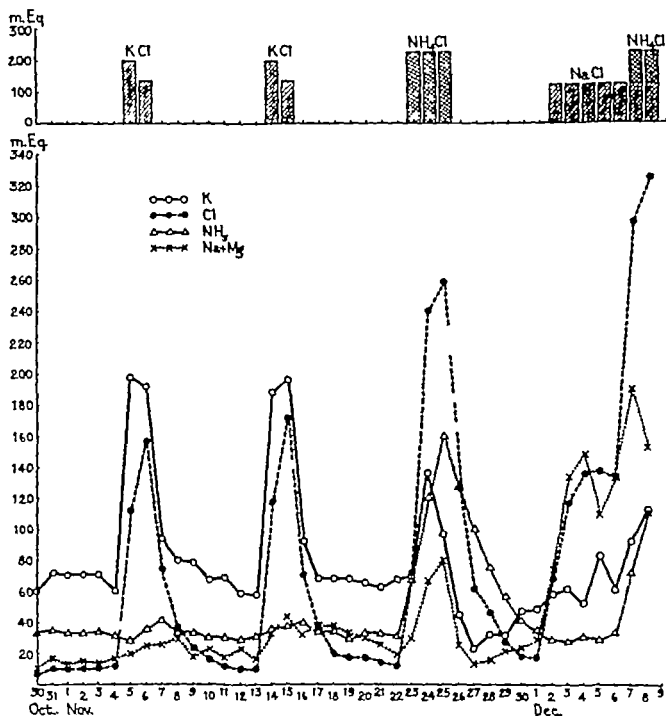


FIG. 2 URINARY RESPONSE OF THE NORMAL SUBJECT TO THE INGESTION OF VARIOUS ELECTROLYTES

KCl there was a slight increase in the excretion of base fraction Na + Mg in the urine. This continued until the extra chloride ingested was completely excreted. While small in amount this base was presumably taken from tissue fluids to neutralize the acidifying action of the Cl ion, as during a period of nine days 10.0 per cent of the K ingested was excreted in the stool daily (exactly as in the foreperiod), whereas Cl absorption was more complete, only 0.5 per cent appearing in the stool each day,

thus increasing the amount to be removed by the kidneys. The lag in excretion of the Cl ion was slightly less in this normal subject than in the nephrotic but otherwise the responses of the two individuals were alike.

In view of the fact that on two occasions in this period the urine specimens were collected in 22 and then 26 hour specimens, they were corrected by the creatinine excretion to a basis of 24 hours for presentation in Figure 2. In order to be certain that this did not vitiate the results, the whole KCl experiment was repeated with essentially the same results. On the first day, 68.2 per cent of the K and only 53.8 per cent of the Cl were excreted. After 48 hours, 79.2 per cent of the K ingested and 77.6 per cent of the Cl had been excreted in the urine. The changes in pH and titratable acid excretion were almost identical with the preceding experiment and the delayed increase in excretion of the base fraction Na + Mg secondary to greater absorption of Cl than K from the gut was again noted. In this instance the daily per cent of K lost through the stools was 12.1 per cent while only 1.0 per cent of the Cl ingested was recovered from the feces. In each of the two experiments with KCl there was a gain of 0.5 kgm. in weight without corresponding retention of sodium during the two days of administration of the salt with a gradual loss to the level of the foreperiod in the following seven days. The reason for this slight gain in weight is not apparent.

*The effect of the ingestion of  $\text{NH}_4\text{Cl}$*  The normal subject was given 224 m. eq. of  $\text{NH}_4\text{Cl}$  daily for three days. The results of this experiment seen in Table 3 and Figure 2 are qualitatively like those observed in the edematous nephritic and essentially like those described by Gamble et al. (20), Følling (21) and others. Thus, there was an increase in the excretion of  $\text{NH}_3$  which in the present study accompanied an increase in the excretion of fixed base (instead of following it as in the case of Gamble's and Følling's studies). The excretion of potassium reached its peak on the second day of  $\text{NH}_4\text{Cl}$  feeding and dropped on the third day, whereas the excretion of the base fraction Na + Mg was slightly higher on the third day. It will be observed above in the study of the patient with edema that there was also slight augmentation in excretion of K and of the fraction Na + Mg + Ca, most marked on the second day. In both the normal and nephritic subjects, retention of the Cl ion on the first day of  $\text{NH}_4\text{Cl}$  administration was marked. There was a great increase in calcium excretion through both the gut and the kidneys, in the normal subject. In the days following the ingestion of  $\text{NH}_4\text{Cl}$ , potassium was retained without appreciable storage of the base fraction Na + Mg while the excretion of  $\text{NH}_3$  and of Cl remained above the normal level until the balances were practically restored on the fifth day. On the first day of  $\text{NH}_4\text{Cl}$  feeding there was a gain of 0.3 kgm. followed by a diuresis and loss of 0.8 kgm. and at the end of the period the weight reached its normal

level (The "recovery" period following the ingestion of  $\text{NH}_4\text{Cl}$  was not studied in the patient with nephrosis)

*The effect of the ingestion of NaCl* One hundred and twenty m eq of NaCl were added to the diet of this normal adult who had been on a "salt poor" regime (Na + Mg of the diet averaged about 45 m eq daily) for seven weeks. In the first forty eight hours he retained 185.4 m eq or 56.6 per cent of the ingested Na + Mg and 181 m eq or 65.1 per cent of the Cl of the diet with a gain of 0.7 kgm in weight. In the next four days of NaCl administration he was in exact balance when compared with the original foreperiod in which there was a positive Na + Mg balance of 15 m eq and a positive Cl balance of 8 m eq daily, probably representing the diurnal skin loss of these ions. The Na and Cl retained during the first two days were not subsequently excreted. The response of this normal subject on the first two days of the "salt regime" was qualitatively like that of the edematous nephritic although the NaCl retention was less complete. It is interesting to note that this similarity of response to NaCl ingestion occurred in spite of the fact that the serum protein concentration in the normal subject was about double that of the nephrotic patient. It should be pointed out that as a result of NaCl administration a rise in the base content of the serum was accompanied by the passage of fluid from the blood to the tissue spaces, as evidenced by the retention of both water and NaCl. This appears to be at variance with the suggestion of Albright and Bauer discussed above, that an increase in base level is associated with the process of diuresis. If this retention of NaCl and water with an increase in concentration of base in the serum could be shown to occur when the serum protein content is abnormally low, the inconsistency would be even more significant. Unfortunately no serum base determinations were made in our nephrotic patient during the period of NaCl administration.

On the last two days of the NaCl experiment, the normal subject was given 224 m eq of  $\text{NH}_4\text{Cl}$  in addition to his daily ration of 120 m eq of NaCl. This was done to see if he would lose fixed base before the augmentation of ammonia excretion, as in the studies of Gamble and of Følling, when an ample source of base was available. It will be seen in Table 3 and Figure 2 that, as in the previous experiments, this subject had a simultaneous increase in the elimination of base and  $\text{NH}_3$ . The total loss of K, Na, Mg and Ca was practically identical when  $\text{NH}_4\text{Cl}$  was given after a prolonged "salt poor" regime with a lowered base concentration in the blood serum and when it was given after sufficient NaCl in the diet to raise the serum base to its normal level.

*The effect of various electrolytes on the blood serum* In Table 4 it will be seen that the "low salt regime" resulted in a decrease in the total inorganic base and Cl concentrations of the blood serum, no other significant deviations from the normal were observed. The administration



TABLE 4  
Blood serum studies on the normal subject

Date	Total base	Potas- sium	B <sub>2</sub> carbo- nate	Chlo- ride	Se- rum pro- tein	Se- rum pro- tein	Phos- phate	Cal- cium	Non- pro- tein nitro- gen	Hae- mato- crit *	Remarks
	m eq per liter	m eq per liter	m. eq per liter	m. eq per liter	per cent	m eq per liter	m eq per liter	m eq per liter	mgm. per 100 cc.	per cent	
November 6	148.9	4.3	30.9	97.4	6.9	16.3	2.7	5.2	29	45.5	Control
November 8	148.7	4.6	28.3	100.0	6.8	16.1	2.6			43.5	After 2 days of KCl feeding
November 14	148.0	4.2	31.2	97.6	6.8	16.1	2.4		32	44.1	Control
November 16	148.8	4.3	27.7	101.0	6.7	15.8	2.6			42.3	After 2 days of KCl feeding
November 26	148.8	3.9	22.2	106.6	6.8	16.1	2.2	5.0	28	42.3	After 3 days of NH <sub>4</sub> Cl feeding
December 1	148.5	4.2	30.9	96.6	6.8	16.1	2.6		25	43.1	Control
December 7	152.3	4.2	30.1	99.3	6.5	15.7	2.6	5.1		43.8	After 5 days of NaCl feeding
December 9	151.0	3.6	23.4	105.7	6.9	16.3	2.2	5.0		41.6	After 2 days of NaCl + NH <sub>4</sub> Cl feeding

\* Determined on whole blood

of KCl in both instances resulted in a slight increase in the Cl content of the serum at the expense of bicarbonate. The feeding of NH<sub>4</sub>Cl resulted in the same qualitative change in the electrolyte pattern of the serum although quantitatively greater, the Cl increasing about 8 m eq per liter while the CO<sub>2</sub> decreased in an equivalent amount. The base concentration and water content were not changed. After six days of NaCl feeding, the concentration of base in the serum rose to the accepted normal level. When the base content of the serum had risen to a more normal value, the storage of NaCl and water in the body ended and the normal balance between ingestion and excretion had been re-established, but at a higher level of base in the serum than in the foreperiod when the patient was on the "low-salt" regime. The effects of NH<sub>4</sub>Cl on the blood serum were qualitatively and quantitatively alike whether the subject received large amounts of NaCl in his diet or whether he was on a "salt-poor" regime.

#### DISCUSSION

At least three groups of factors must be taken into consideration in any discussion of the mechanism of edema in the nephrotic form of chronic nephritis and in malnutrition. (1) The best understood of these is the decrease in osmotic pressure of the blood due to loss of proteins from the serum. (2) The second factor is that of the hydrostatic pressure differences between the capillaries and the tissue spaces. (3) The third factor and the least understood is that of the specific ionic excretory function of the kidney. In this term we include all the factors involved in the elimination of specific ions from the body, recognizing that such elimination seems to depend upon the particular nature of the ion itself and is largely independent of its osmotic effect. In other forms of edema variations in capillary permeability undoubtedly play an important rôle,

but it does not appear necessary in the present state of our knowledge to implicate this factor in the explanation of nephrotic edema. As was stated in the introduction there have existed two schools of thought. One emphasizes the importance of specific kidney or tissue disturbances as evidenced by an apparent difference in behavior between the nephrotic and the normal individual upon the administration of certain electrolytes. The other group of investigators maintains that the abnormal accumulation of fluid and the behavior of the electrolytes can be explained solely on the basis of a decrease in oncotic pressure and assumes that the ionic excretory functions of the kidney are normal.

If it be assumed that the specific ionic excretory functions of the kidney are not disturbed in patients with nephrotic or nutritional edema, it is apparent that a decrease in oncotic pressure within the capillaries will tend to force an approximately normal intercellular fluid into the tissue spaces until the effective pressures and ionic concentrations on both sides of the capillary adjust themselves according to the Donnan equilibrium. It is obvious that an important factor in the accumulation of this excessive volume of interstitial fluid is the quantity of water and NaCl that is available, therefore the administration of NaCl to an individual with a decreased concentration of protein in his blood serum will cause an increased flow of physiological salt solution to the tissue spaces. This retention would naturally simulate interference with the ability of the patient to excrete NaCl. A state of affairs qualitatively similar, might be anticipated in a normal individual after the prolonged restriction of NaCl ingestion, resulting here from true base depletion. Furthermore, one would not expect to find a qualitative difference in response of the normal and nephrotic individual to the ingestion of KCl or  $\text{NH}_4\text{Cl}$ , as the nephrotic is assumed according to this hypothesis to possess normal specific ionic excretory functions.

The experiments described in this paper amply confirm this point of view. Thus, the nephrotic patient retained NaCl completely with a coincident increase in edema as evidenced by gain in weight. The normal subject after prolonged NaCl deprivation also retained salt and water for the first two days of sodium chloride feeding. The quantitative difference in the behavior of these two individuals can be easily explained by the difference in oncotic pressure of their sera. Furthermore, our studies show that there is no essential qualitative difference in the response of these two individuals to the ingestion of KCl or of  $\text{NH}_4\text{Cl}$ .

The facts presented in the foregoing discussion do not support the idea chiefly advanced by the school of Widal, Magnus Levy and of Blum, that the determining abnormalities in nephrotic edema are to be found in specific ion disturbances of renal or tissue behavior.

The importance of the hydrostatic pressure within the capillaries in relation to edema needs no emphasis. Von Farkas (22), Mufson (23)

and others have pointed out a relationship between the concentration of protein in the blood serum, the venous or capillary pressure in an extremity and the presence of edema. As would be expected, there appears to be a rough correlation which would suggest that the higher the pressures within the veins or skin capillaries, the more likely a patient is to become edematous with a given decrease in the serum protein concentration. Unfortunately, these measurements are of the crudest sort and are applicable only to a limited group of capillaries. Beyond these elementary facts, little is known of the relations of capillary pressures to edema of the nephrotic type.

While the qualitative similarity in response of the nephrotic and normal subjects to the ingestion of certain electrolytes has been demonstrated in the above studies, it should be clearly emphasized that the mechanism of nephrotic edema cannot be explained solely by alterations in the concentration of protein in the blood serum. For example, as was stated in the introduction and as we have observed in the nephrotic patient of the present study, there may be complete diuresis without significant change in the concentration of protein in the serum. Furthermore it is well recognized that diuresis may occur spontaneously or follow fever either induced artificially or due to infection.

In addition to these observations, apparently inconsistent with the hypothesis, certain theoretical difficulties should be mentioned. If a decreased oncotic pressure causes increased flow of fluid from the capillaries to the tissue spaces in the kidneys, one might expect increased filtration through the glomerular capillaries and *ceteris paribus* an increased urinary output, which is not the case. If, however, it were assumed that, in conditions leading to nephrotic edema, the rate of blood flow through the kidney or the capillary pressure in the glomeruli were reduced or if the reabsorption of water from the tubules were increased, these discrepancies could be harmonized with the theory. Of such changes and the possibility of actual renal damage we have no experimental evidence.

Another phenomenon for which the oncotic hypothesis offers no explanation is the well known diuretic effect of certain electrolytes such as potassium salts,  $\text{NH}_4\text{Cl}$ , and  $\text{CaCl}_2$ . Although there is no qualitative difference in the behavior of the electrolytes  $\text{KCl}$  and  $\text{NH}_4\text{Cl}$  when ingested by normal and nephrotic individuals, as shown by our studies, there may be great quantitative differences in the effect of these substances on various edematous individuals and upon the same individual at different times. While these differences have been ascribed to differences in base concentration in the blood, this point is not yet proved, there is also no simple correlation between diuresis and serum protein concentration. This problem is closely related to that of the kidney or tissue factors which regulate the excretion of the  $\text{Na}$  ion by the kidney. Until the physicochemical laws governing the excretion of various ions

are understood, an evaluation of abnormalities present will remain difficult, since normal and nephrotic individuals may react to these ions in a *qualitatively* similar manner

#### CONCLUSIONS

1 The responses of a normal individual on a "salt-poor" regime and of a patient with nephrotic edema to the ingestion of KCl,  $\text{NH}_4\text{Cl}$  and NaCl have been studied in balance experiments

2 The following similarities in their behavior have been demonstrated

(a) When KCl was fed to both patients the urine at first became alkaline, as the excretion of K was more rapid than that of Cl. Then the urine became more acid and there was an increase in the excretion of  $\text{NH}_3$  and fixed base. This probably resulted from the fact that the absorption of Cl from the intestinal tract was greater than that of K, KCl thus acting as an "acidifying salt."

(b) After feeding  $\text{NH}_4\text{Cl}$  there was an increase in excretion of inorganic base and  $\text{NH}_3$  with retention of Cl causing a lowering of serum bicarbonate, as has been described by others. No significant diuresis occurred in these patients

(c) After ingesting NaCl, both the nephrotic patient and the normal subject retained NaCl and water. This retention was greatest in the edematous patient. After the base of the blood of the normal subject on a "salt poor" regime had been raised to its normal level by NaCl administration, continued ingestion of this salt caused no further retention

3 It is pointed out that the responses of nephrotic and normal individuals to these electrolytes are *qualitatively* alike. The *quantitative* differences observed are of great clinical importance and are probably to a large extent dependent upon differences in serum protein concentration

4 A number of inconsistencies in the application of the oncotic hypothesis are discussed

The authors wish to express their appreciation to Miss Natalie Bryan, Miss Marjorie Clark and Miss Evelyn Krueger for their technical assistance. They also wish to thank Miss Margaret Hawthorne and Miss Elise Stanley for their careful supervision of the nursing and dietetic problems arising in the course of this investigation

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# THE EFFECT OF VENTRICULIN ON THE BLOOD SUGAR LEVEL IN PATIENTS WITH PERNICIOUS ANEMIA

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A reduction of the blood sugar content, analogous to that produced by insulin, was noted by Blotner and Murphy (1) after administration of whole liver and certain of the liver fractions ineffective in the treatment of pernicious anemia. Fractions G, an effective liver extract prepared by Cohn and his collaborators (2), appeared to contain little if any blood sugar reducing substances. An observation repeated by Riddle (3) substantiated the evidence that this liver extract contained no specific blood sugar reducing properties. After the introduction by Sturgis and Isaacs of ventriculin as a specific treatment in pernicious anemia (4), it was decided to determine whether or not this preparation possessed blood sugar reducing properties similar to those of whole liver.

The blood sugar level was determined on eleven patients with pernicious anemia at frequent intervals during treatment with ventriculin, liver extract, and on one patient in spontaneous remission. The samples of blood were obtained under fasting conditions at 8 A M, and the blood sugar values were estimated according to the micro method of Folin and Wu (5). Each patient was given 40 grams of ventriculin daily, and observations were made during the interim of the rise of the reticulated red blood cell count, at intervals of one to two days. Data on the red blood cell counts, hemoglobin and reticulocyte percentage were also recorded.

The fasting blood sugar values obtained during the treatment with ventriculin are listed in Table I. The results for each patient are recorded separately, showing daily variations as well as the general trend. With two exceptions, the initial values were within the range of 84-135 mgm of sugar per 100 cc of blood—the majority of the results bordering on the upper limits of normal. Six to ten days after the onset of treatment, the blood sugar values in each case reached their lowest levels, the decrease being gradual. These minimal readings were within the range of 61-97 mgm. The average decline of the blood sugar level was about 45 mgm per cent, the upper and lower extremes being 30 and 60 mgm. In the series of cases which were studied during the entire period of the rise and fall of the reticulated cell count, the blood sugar levels



TABLE I  
*Milligrams sugar per 100 cc of blood*

Days of treatment	Ventriculin treated patients											Spontaneous remission
	Patient number											
	1	2	3	4	5	6	7	8	9	10	11	
	mgm	mgm	mgm	mgm	mgm	mgm	mgm	mgm	mgm	mgm	mgm	
-2			92		138	109		124	84			
-1	131	107	99	135		115	118	115		94		
0	110	102	85	130	135	137	120	109	90		114	
1	137	107	85	120	120		117	138	78	96	108	
2	110			143	138	105	117	121	85	95		
3			75	125	151	102	103		87	94		133
4	111	98	68		135	97	101	131	81	94	117	
5	103	95	75	106	106	100	105	125		89		91
6	95	104	70	105	95	111	93	121	75	93	100	
7	100	83	61	107	94	83		97	80	85	95	91
8	104	80		93	105	72				95	105	
9	143	78		86	94							101
10	108	75				95				89		
11	97.5	84		102	100	102						103
12		111			117					95		
13	121	109		114		105						
14		148			109							
15	121	118		108	115	125						

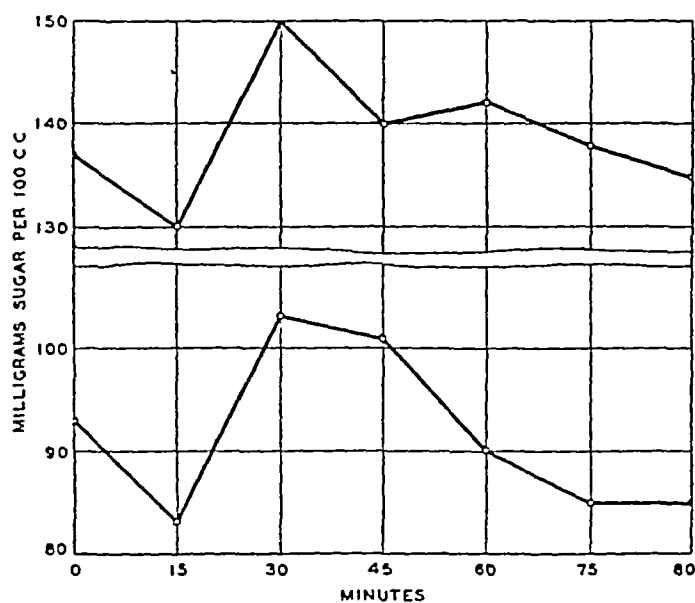


FIG 1 EFFECT OF INGESTION OF 40 GRAMS OF VENTRICULIN ON BLOOD SUGAR LEVEL OF TWO PATIENTS WITH PERNICIOUS ANEMIA

reached their lowest values at the time of the peak of the reticulated red blood cell percentage rise, subsequently undergoing a gradual upward swing but not quite attaining their initial values in the majority of cases.

In two cases an attempt was made to determine any immediate effect of the ingestion of ventriculin on the blood sugar level. Blood samples were taken before and at intervals of 15 minutes for one and a half hours after the administration of 40 grams of ventriculin. These results are shown in Figure 1. The first specimens showed a slight decrease of 7-10 mgm, followed by an increase which attained its maximum in the one-half hour specimens. The values declined to approximately their original levels within one hour. The immediate effect of the administration of 40 grams of ventriculin was a slight increase in the blood sugar level, similar to that occurring after the ingestion of any other similar food substance.

Following treatment with ventriculin, as with liver extract, a marked increase of appetite was noted in each of the cases. For 2-4 days after the onset of treatment with ventriculin, no change in the appetite was observed, however, with the beginning of the reticulocyte response, a noticeable increase was evident. The usual diet which was more than sufficient during the first few days was supplemented with extra nourishment. Often the patients were given high caloric diets to appease their hunger. Inasmuch as the majority of the patients often lose weight in early remission, due probably to water loss (6), the weight factor could not be used in correlation with the hunger symptoms.

As the symptoms of hunger seem to be associated with both the increase of reticulated red blood cells and the amount of sugar present in the blood, the correlation of these two values is of interest. Determinations of the reticulocyte percentage and blood sugar values of three cases are recorded in Table II. Case 1 was treated with ventriculin, Case 2 with liver extract. Each was selected as representative of a series of ten cases similarly treated. Case 3 is one of pernicious anemia in spontaneous remission. If the blood sugar values are plotted together with the reticulated red blood cell percentage of the same day (Figures 2 and 3), it is noted that the lowest blood sugar level occurs simultaneously with the peak of the reticulocyte percentage rise. Apparently this behavior is physiologic, as it is always present regardless of the type of treatment, or if the remission is spontaneous. It is fair to conclude from this that the reduced sugar level is the result of increased metabolic activity of the bone marrow, as manifested by the increase of young red blood cells in the stream, or, that the reduced sugar values are the result of the increased metabolic activity of the young red cells themselves, or both. Riddle (3) stated that the cause for the lowered fasting blood sugar values during early remission was speculative, and that the evidence pointed to some unknown metabolic process. This process is now thought to be

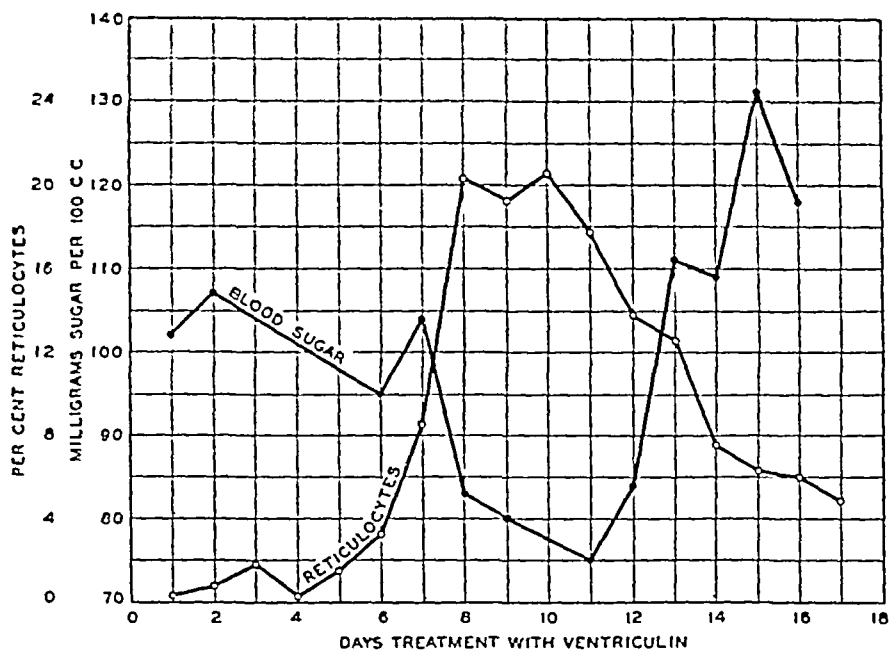


FIG 2 RELATIONSHIP OF BLOOD SUGAR AND RETICULOCYTES FOLLOWING VENTRICULIN THERAPY

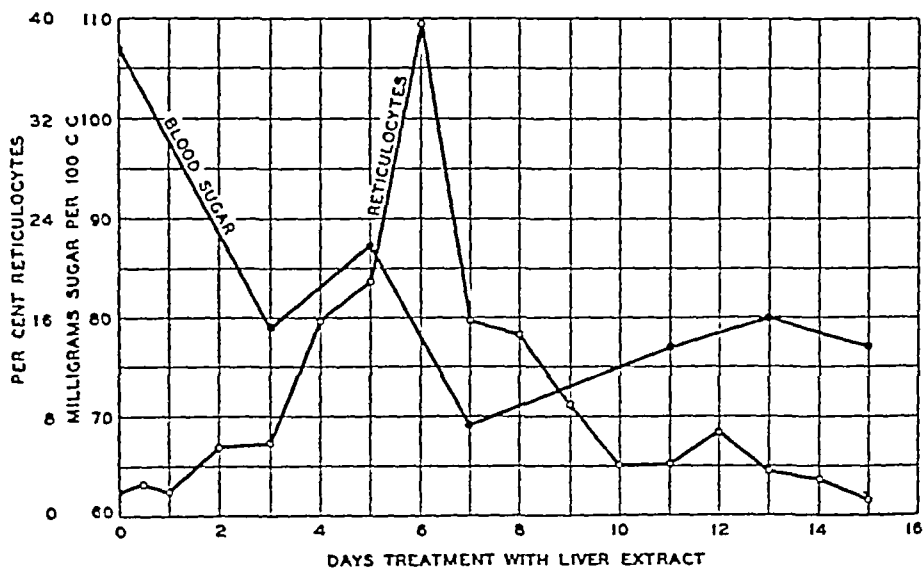


FIG 3 RELATIONSHIP OF BLOOD SUGAR AND RETICULOCYTES FOLLOWING LIVER EXTRACT THERAPY

TABLE II

*Fasting blood sugar values during treatment with ventriculin, liver extract and spontaneous remission*

Days of observation	Case 1*		Case 2*		Case 3*	
	Blood sugar	Reticulocytes	Blood sugar	Reticulocytes	Blood sugar	Reticulocytes
	mgm. per 100 cc.	per cent	mgm. per 100 cc.	per cent	mgm. per 100 cc.	per cent
-4			93	2.3		
-3			88	1.4		
-2				2.0		
-1	107	.8	107	2.1		
0	102	.2		2.5		
1	107	.8		2.0		7.5
2		1.9		5.2		10.2
3		.3	79	5.7	133	17.9
4	98	1.5		15.9		18.4
5	95	3.2	87	18.0	91	11.9
6	104	8.5		39.5		9.6
7	83	20.1	69	15.1	91	9.6
8	80	19.2		14.6		7.3
9	78	20.3		9.0	101	8.0
10	75	17.6		4.3		6.8
11	84	13.8	77	4.3		5.5
12	111	12.1		5.8	103	2.2
13	109	7.5	80	3.8		
14	148	6.4	80	3.1		
15	118	6.0	77	2.2		

\* Case 1—40 grams ventriculin daily

Case 2—6 vials Lilly's liver extract daily

Case 3—Spontaneous remission

the increased activity of immature red blood cells, of the bone marrow, or a combination of the two

The results obtained have been made from observations, which disregard the blood volume factor. The influence of the blood volume on the concentration of the blood sugar levels is not known at this time. However, its importance and significance are being studied

#### SUMMARY AND CONCLUSIONS

1 In pernicious anemia in relapse, the blood sugar values are usually within the upper limits of normal

2 The decreased blood sugar values in the beginning of a remission occur at the time of the increase in the percentage of immature red blood cells present in the peripheral circulation

3 The blood sugar level reaches its lowest value at a time corresponding with the peak of the reticulated red blood cell percentage rise.

4 In early remission the blood sugar level may fall as low as 61 mgm

per 100 cc—practically all the values were within the lower limits of normal

5 The onset of hunger symptoms in early remission may be directly associated with the decrease in the blood sugar level—the result of increased metabolic activity of either the marrow, the immature red blood cells, or both

6 With general improvement, the blood sugar values increase and maintain themselves at a level not quite as high as their original one

7 Ventriculin has no specific insulin-like properties when taken by mouth—the immediate effect following ingestion of 40 grams is a slight increase in the blood sugar value for about one-half hour, with a gradual return to its original level

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# THE EFFECT OF INJECTION OF NONSPECIFIC PROTEIN ON THE PAIN OF ULCER AND ON GASTRIC SECRETION A CLINICAL AND EXPERIMENTAL STUDY

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The search for additional forms of treatment for peptic ulcer refractory to the usual medical and surgical treatment has led to the introduction of many innovations. Among these is the injection of nonspecific protein, first introduced by Holler in 1921 (3) and later advocated by Pribram (9) and other investigators in Germany. Martin (4), Shaine (11), Meyer and Kartoon (6), and Schiff and Norris (10) have adequately reviewed the literature on this subject and outlined the development of this method of treatment. Briefly, it has been found that in cases of peptic ulcer symptoms of ulcer disappear completely for a variable length of time following the parenteral injection of various protein substances. Reports are at variance as to the percentage of patients who obtain relief, the duration of symptomatic improvement, and the probable effects of the procedure on the healing of ulcer. Also, little is known about the effects of the injection of foreign protein on gastric secretion and motility in normal and diseased human subjects. This paper sums up the results of work which we have done in an attempt to answer all these questions and in addition to determine the effects of the procedure on the gastric motor and secretory functions of dogs.

## CLINICAL OBSERVATIONS

The difficulty of evaluating any treatment for peptic ulcer lies in the nature of the disease itself, with its great tendency to spontaneous remission and the almost equally great tendency to recurrence. Many observers have testified to the occurrence of symptomatic relief following injections of nonspecific proteins. Although such relief has been noted in intractable cases, the statement is often made that acute ulcers of short duration are more responsive to treatment by nonspecific protein than are chronic callous ulcers. In other words, good results are obtained in the group of cases in which the best results would be obtained by any treatment, and in which spontaneous remissions are most likely to occur even

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without treatment. In an attempt to avoid the possibility of assuming that what was really a spontaneous remission was due to our efforts at treatment, we studied only such patients as had had for some time symptoms so severe and intractable that spontaneous improvement was not likely to take place. None of the group of seventeen patients selected had improved materially under previous medical treatment. Moreover, all of them had had some form of surgical treatment, that is, they had submitted to one or more operations on the stomach. In every instance the diagnosis was based not only on a typical history but on a roentgenologic demonstration of a lesion, and in nine instances an ulcer was later demonstrated at operation. Four of the patients had recurrence of duodenal ulcers following operations on the pylorus, one patient had a large gastric ulcer persisting after gastro-enterostomy, and twelve patients had anastomotic ulcers. One of these twelve patients had recurrent ulcer following anterior gastro-enterostomy, six patients had ulcer following posterior gastro-enterostomy, and five, after partial gastric resection.

The ages of the patients ranged from twenty-four to fifty-one years, the average age was thirty-seven and five-tenths years. The average age at onset of dyspepsia was twenty-three and a half years, the youngest patient had had symptoms since the age of seven years. The duration of symptoms varied from five to twenty-four years, averaging fourteen years. Most of the patients had suffered severe symptoms and in every case the course of the disease was marked by many complications. Both medical and surgical treatment had been followed by indifferent or poor results. Thirteen of the patients had required more than one operation, of these two had required four operations each and one had required five operations.

#### METHODS

Many proteins have been used by various experimenters, but those most frequently employed have been a purified vegetable protein, known as "novoprotein," suspensions of killed bacteria, chiefly typhoid vaccine, and various sterilized and purified preparations of milk. There is no uniformity of opinion as to the degree of general reaction necessary to produce the maximal amount of benefit. We used Lederle's triple typhoid vaccine or Lilly's type "H" antigen intravenously in doses containing from 10,000,000 to 60,000,000 bacteria. An effort was made to secure a sharp reaction lasting a few hours with elevation of temperature not exceeding 3° F. Usually four to six injections were given at intervals of three or four days. In a few instances 10 cc. of a purified preparation of milk (aolan) was given intramuscularly, a milder but definite reaction being produced by this means.

Before the injections of foreign protein were started, the patients were placed on a modified Sippy treatment for variable periods of time. After injections were begun changes in diet were not made until clinical

improvement was noted. If, and when, improvement occurred the diet was made more liberal, but in no instance were the usual dietary restrictions exceeded that were considered necessary for the ambulant treatment of patients with ulcer.

Studies were made of the fasting contents of the stomach each morning for several days before the treatment was begun and daily thereafter to determine the influence of the treatment on gastric acidity. Besides the alcohol test meal, as devised by Bloomfield and Keefer (1), the Ewald test meal was used in a few instances before and after treatment to determine any change in secretory function.

### RESULTS

Fourteen of the seventeen patients were relieved of symptoms of which pain, of course, was the most prominent, whereas the remaining three patients suffered marked aggravation of symptoms (Table 1). One of these three patients had duodenal ulcer associated with marked hypersecretion, one had an extensive penetrating gastrojejunal ulcer, and one had a poorly functioning gastro-enteric stoma and also gastric, duodenal, and anastomotic ulcers. Of the fourteen patients who experienced amelioration of symptoms, four had some degree of relief lasting for several days after each injection. One of these patients had a gastric ulcer which when observed fluoroscopically appeared to increase rapidly in size during the period of subjective improvement. One patient was so completely relieved of symptoms that he was allowed to return to his home; unfortunately the later course of the disease is unknown. Although two other patients were relieved of pain, the severity of the general reaction and the associated mental and emotional depression produced by the injections made them unwilling to continue the treatment. Four of the patients had a remission of one or more months before symptoms recurred: one had symptoms of pyloric obstruction three months later, one was relieved for several months when a second course of vaccine produced a similar period of remission, but epigastric pain and nausea have continued at intervals. The two other patients were relieved for about one month, one of these responded well again to a second course, but later returned with severe symptoms and a deep perforating jejunal ulcer, the other was made worse by a second course of vaccine, and at operation a large crater was found in the jejunum which had almost perforated into the colon.

The three remaining patients obtained more gratifying results. One was a young man who had symptoms of obstruction at the gastro-enteric stoma due to jejunal ulcer, the crater of which was visualized in the roentgenogram. He had little pain and no symptoms except those associated with obstruction. On a liquid diet, gastric retention of about 1,500 cc. of highly acid fluid was noted. After the first injection of



vaccine the amount of retention decreased markedly in spite of the fact that the amount and the coarseness of the food given were steadily increased. The fasting acidity dropped to an average value of 14 units and the stomach emptied normally. Several months later when there was a return of mild symptoms of obstruction vaccine was again given with good results. Since then the patient has had recurrent periods of obstruction which are relieved spontaneously, and in general he has remained reasonably well.

The second patient was a woman with mild and questionable symptoms of ulcer but a definite roentgenologic picture of gastrojejunal ulcer. Following two intravenous injections of typhoid vaccine she was almost entirely relieved of distress. In a week's time she gained 5 pounds, and

TABLE 1

*Reaction before, during and after injection of nonspecific protein (clinical data)*

Case	Age and sex	Ulcer	Treatment	Free acidity in content of fasting stomach			Results
				Before	During fever	After	
1	34 M	Gastrojejunal	2 courses of vaccine	cc N/10 per 100 cc 92	cc N/10 per 100 cc	cc N/10 per 100 cc 13	Relief of obstructive symptoms
2	33 M	Duodenal	4 injections of vaccine	18	0	40	Relief for at least eight months
3	40 F	Gastrojejunal	2 injections of vaccine	30		0	Improvement persisting for at least two years
4	35 M	Gastrojejunal	4 injections of vaccine	32		61	Relief during treatment, later course unknown
5	35 M	Gastrojejunal	2 injections of vaccine	30	16	52	Symptomatic relief, but refused further treatment
6	70 M	Gastrojejunal	2 injections of vaccine				Pain relief, further treatment refused
7	45 M	Duodenal	4 injections of vaccine	64		56	Symptomatic relief for three months, then developed symptoms of obstruction
8	33 M	Duodenal	2 courses of vaccine	20	4	58	Remission followed each course
9	51 M	Gastrojejunal	2 courses of vaccine	56	18	12	Prolonged relief from pain but less on progressive

TABLE 1 (continued)

Age and sex	Ulcer	Treatment	Free acidity in content of fasting stomach			Results
			Before	During fever	After	
years			cc. N/10 per 100 cc.	cc. N/10 per 100 cc.	cc. N/10 per 100 cc.	
37 M	Gastrojejunal	2 courses of vaccine				Complete relief for one month from first course none from second course
39 M	Gastrojejunal	5 injections of vaccine	30	35	62	Relief from pain for a few days after each injection
47 M	Gastrojejunal	4 injections of vaccine				Temporary relief
42 M	Gastrojejunal	2 injections of vaccine and 3 of aolan				Complete relief from pain for a few days
50 M	Gastric	1 injection of aolan and 2 of vaccine	67	45	84	Temporary partial symptomatic relief but ulcer increased in size
35 M	Duodenal	3 injections of vaccine	44	34	35	Aggravation of symptoms
29 M	Gastrojejunal	2 injections of vaccine				Marked aggravation of symptoms
24 M	Gastrojejunal duodenal and gastric	5 injections of vaccine				Aggravation of pain

able to return to her home. Two years later she reported that she was not entirely well but was much better than she had been. At a later visit to the clinic it was decided that the remaining symptoms could be attributed to ulcer.

The third patient was a man who had had four operations on the stomach, the last one a pyloroplasty. At the time of admission he was having severe pain which suggested the presence of a perforating type of duodenal ulcer. After five injections of typhoid vaccine he was relieved satisfactorily and was able to return to work. Eight months later he reported that he was still free from distress although he found it necessary to continue on a restricted diet.

## COMMENT

It would seem from the foregoing that the results of protein treatment are usually inconstant and temporary although occasionally a patient will show a striking and fairly permanent improvement. In order to produce these results, it unfortunately is necessary that the patient be made to suffer more or less discomfort, sometimes so much that the treatment is more harrowing than the disease. Occasionally the temperature will rise to  $104^{\circ}\text{F}$  and the patient will be utterly miserable for twelve hours or more. At other times the reaction is delayed, the fever is prolonged and undesirable by-effects are likely to make their appearance.

An effort was made to determine which type of reaction was most likely to be helpful in relieving the symptoms of ulcer. A febrile response of less than  $100^{\circ}\text{F}$  rarely produced any definite effect. If reactions were accompanied by fever of more than  $103^{\circ}\text{F}$  the symptoms of ulcer were often aggravated. An abrupt febrile response of two or three hours' duration with a maximal temperature of  $102^{\circ}\text{F}$  was least disagreeable to the patient, and seemed most likely to produce relief from the symptoms of ulcer.

Of the fourteen patients who were benefited, twelve obtained relief from the first injection. This relief was often spectacular and patients who had been taking milk or alkali at frequent intervals throughout the night and who in addition had been taking opiates to control the pain were often completely relieved of distress within a few hours. Unfortunately, this relief was rarely permanent, the symptoms usually returning after a few days or weeks.

In the first few cases studied, we found what appeared to be a definite reduction in gastric acidity but in the larger group of cases studied later there did not seem to be any response to the treatment. The acidity of fasting contents removed during the course of a febrile reaction and while the temperature was still elevated was studied in six cases. The acid values were practically unchanged in two and distinctly lowered in four. In one case, the acidity was increased threefold a few hours after the termination of fever, a result comparable with that seen in our experimental animals. In all this work, titrations of fasting contents were made for a few mornings before treatment was instituted and then daily after the treatment. The readings obtained during the two periods were averaged separately so as to give a more trustworthy index of the conditions actually existing.

Similar studies of gastric juice obtained after the use of alcohol and Ewald meals showed the same absence of any constant change in acidity following the treatment.

There was no demonstrable correlation between the reduction of acidity and the degree of symptomatic relief obtained. In one instance,

a rise of acidity from an average of 67 units before treatment to an average of 84 units after treatment was associated with relief of symptoms, later, after another injection when the acidity dropped to 63 units the pain became worse. It should be noted, however, that two of the three patients (Cases 1 and 3 in Table 1) who received the greatest benefit from the treatment showed the most marked decrease in acidity observed after the febrile reactions. Another patient who showed a considerable drop in acidity was relieved of symptoms after each of two courses of treatment, but ultimately had to resort to operation (Case 9)

#### EXPERIMENTAL OBSERVATIONS

When, with the first few cases studied, the gastric acidity seemed consistently to be reduced we became hopeful that the relief of symptoms was due to this change and that the use of vaccine had some possibilities in the treatment of ulcer. In an effort to learn more about this change in acidity experiments were begun in animals.

#### METHOD

Healthy young dogs of medium size were chosen for study. Aspiration of gastric contents was made, using a lavage tube passed in the same manner as in human subjects except that a wooden mouth gag was necessary to hold the jaws open. Aspirations were practiced repeatedly, day after day, until there was no longer any emotional reaction to the procedure. Fractional tests were then made several times a week over a period of many weeks until the curves for acidity on successive days were practically identical (Figure 1). More than 2,000 tests were made during the period of training, and 1,000 tests were made during the experimental period.

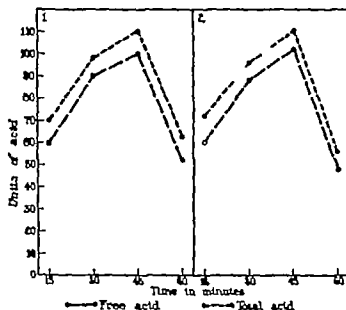


FIG 1 GASTRIC SECRETION OBTAINED FROM A NORMAL DOG  
ACID IN CC N/10 PER 100 CC.

The uniformity is shown of repeated tests possible in well trained animals. Histamine injected 1 at 8:30 a.m., and 2 at 11:00 on the same day.

The test meal which consisted of 80 grams of finely ground horse meat in 250 cc. of water, was fed in the morning after twelve hours of fasting. Small specimens were removed every fifteen minutes after the first half hour until the stomach was empty. The clear juice from a centrifuged sample of the contents was then titrated with 0.1 normal sodium hydroxide, using dimethyl-amino-azobenzene and phenolphthalein as indicators to determine free and total acids, respectively. The hydrogen-ion concentration was determined by means of a Leeds-Northrup potentiometer, using a quinhydrone electrode.

The gastric secretory response to histamine was also used as a measure of gastric function. For this test the stomach was aspirated as completely as possible every fifteen minutes following the injection of 1 mgm. of histamine subcutaneously.

To produce febrile reactions, various foreign proteins were given, but the intravenous injection of a suspension of a killed culture of *Bacillus prodigiosus* proved to induce fever more uniformly than any of the other substances employed. An initial dose of 100,000,000 organisms was used and each subsequent dose increased by an amount which depended on the severity of the previous reaction, the object being to produce a temperature of about 104° F.

As a rule the rectal temperature rose from normal of about 100° to 104° F. or more within two hours after the injection. While the fever was at its height, the gastric secretion was studied following stimulation with histamine since it was impossible to induce the dogs to take a test meal of meat during the febrile stage. When the fever subsided, which it did within two or three hours, the dogs had good appetites and were then fed their usual ration. The following morning the gastric secretion was again examined, using either histamine stimulation or a test meal of meat.

Bilateral intrathoracic section of the vagi had been done on three of the dogs two years before, and a fourth dog had had the branches of the vagus nerves sectioned below the diaphragm. The latter animal subsequently had the vagus nerves sectioned again within the thorax. Three additional dogs were operated on after a period of observation of their response to fever. Under ether anesthesia, administered intratracheally, with careful aseptic technic, both vagus nerves of one of them were cut in the thorax and intrathoracic portions of both splanchnic nerves were removed from the other two. After they had recovered from the operation, the response of gastric secretion to fever was again studied.

## RESULTS

Dogs were found to vary markedly in susceptibility to fever. In two of the nine dogs used it was impossible to produce an adequate febrile reaction; it was readily produced in the remaining seven. During the

course of the fever the dogs almost invariably appeared listless and some times they vomited. As soon as the temperature began to fall, they speedily recovered. The secretory response to histamine during the time that the temperature was rising or was at its peak was usually markedly subnormal, both as to volume of secretion and acidity (Figure 2). Often only a few cubic centimeters of thick bile-stained mucus could be obtained in which free acid could not be demonstrated. Rarely, and particularly when injections of protein were repeated at intervals of less than three days, both the volume and acidity of the gastric juice were only slightly reduced. Occasionally the stomach was filled with a large amount of fluid which had probably regurgitated from the intestine be-

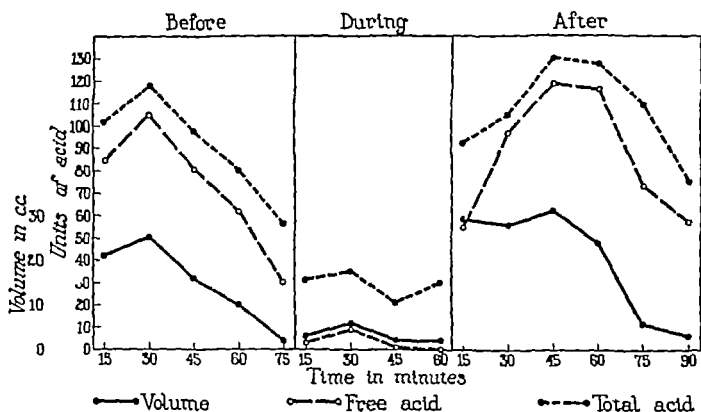


FIG 2 THE VOLUME AND THE TOTAL AND FREE ACIDITY IN CC N/10 PER 100 CC OBTAINED FROM A NORMAL DOG FOLLOWING HISTAMINE GIVEN BEFORE, DURING, AND THE DAY AFTER REACTION TO PROTEIN SHOCK

cause it had an almost neutral reaction and contained large amounts of bile. In dog 2 (Table 2) the usual reduction in acidity during the reaction did not occur.

The decrease in secretion usually seemed to depend on the fever, because, except in Dog 1, it never altered until the temperature began to rise. Although in Dog 1 the temperature never rose higher than 102° F the animal would appear ill, and free hydrochloric acid would either be absent or very much reduced during a period of from four to six hours.

We can give some idea of the inhibition of gastric secretion produced by the fever by saying that all the juice that could be obtained after the injection of histamine during the febrile period could usually be neutralized by 12 cc or less of 0.1 normal sodium hydroxide.

TABLE 2

Secretory response during and after reaction of a specific protein (experiment 1 day)

Animal		Volume			Maximal acidity			Total hydrochloric acid obtained		
		Before	During	After	Before	During	After	Before	During	After
Dog 1	Normal	74	13	121	105	9	119	64	1	114
Dog 2	Normal	85	77	131	77	91	98	56	49	94
Dog 3	Normal	58	36	76	90	62	101	43	12	81
	After cutting splanchnic nerve	58	25	70	77	16	90	37	1	56
Dog 4	Normal	70	22	100	97	27	107	57	8	81
	After cutting vagi	55	12	18	51	0	32	20	0	9
Dog 5	Normal	58	24	97	88	27	81	44	5	62
	After cutting splanchnic nerve	73	35	83	79	20	80	49	5	51
Dog 6	Two years after cutting vagi in abdomen	66	68	90	60	5	96	32	2	71
	After cutting vagi in thorax	77	10	68	50	0	32	22	0	19
Dog 7	Two years after cutting vagi in thorax	33	20	49	72	13	92	19	3	31
Dog 8	Two years after cutting vagi in thorax	28	18	75	53	15	98	12	2	59
Dog 9	Two years after cutting vagi in thorax	37	45	43	60	12	72	20	2	28

On the day following the reaction to protein, the response to histamine was always as great as during the control period and in most instances it was definitely increased (Figure 3). Although only three animals secreted more than 50 cc. of 0.1 normal acid under normal conditions, nine of them exceeded this amount after a reaction (Figure 4 and Table 2). The level of free acid after a test meal of meat was likewise greatly elevated in the postfebrile period.

The period during which this increased secretory response could be obtained was brief. As a rule normal values for gastric acidity were obtained after forty-eight hours, although when injections of vaccine were repeated over two or three days the return to normal was retarded.

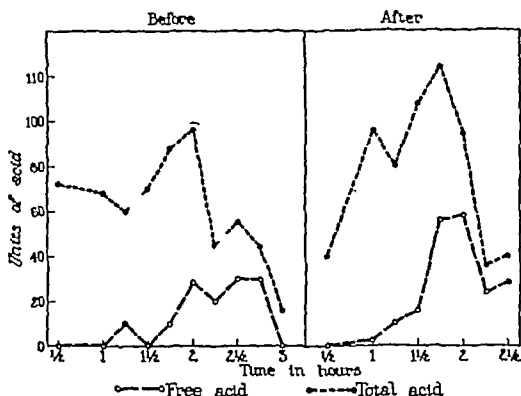


FIG 3 THE TOTAL AND FREE ACIDITY IN CC N/10 PER 100 CC AFTER TEST MEALS OF MEAT GIVEN THE DAY BEFORE AND THE DAY AFTER REACTION TO PROTEIN SHOCK

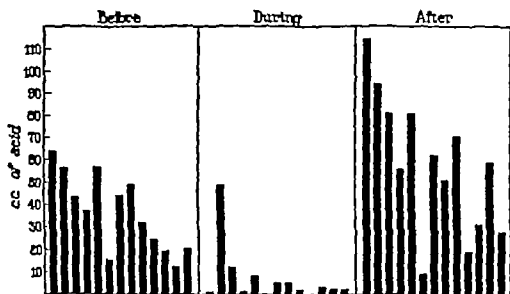


FIG 4 THE AVERAGE AMOUNTS OF HYDROCHLORIC ACID EXPRESSED AS CUBIC CENTIMETERS OF 0.1 NORMAL ACID OBTAINED IN EACH DOG BEFORE, DURING, AND AFTER REACTIONS TO PROTEIN SHOCK

The same order of presentation of data is used as in Table 2. Thus, the first bar in each of the three sections represents the first dog, the second bar represents the second dog, and so forth.

#### COMMENT

From these results it is apparent that the effect on gastric secretion of intravenous injections of foreign protein is biphasic with first a lessening of the juices and later an increased amount of fluid and acid.

An attempt was made to learn if their reactions were due to a direct effect of the protein injected on the secretory cell itself or an indirect one by way of the nervous system. In Nonnenbruch's opinion (7) the reac-





Only three of the seventeen patients treated obtained relief which lasted for some time while four had temporary relief. Four of the patients experienced such brief periods of relief and the febrile reactions were so unpleasant that it did not seem advisable to continue the treatment. The condition of the ulcer of three patients grew worse during the period of treatment and observation, although all of these obtained some symptomatic relief. The symptoms of the remaining three patients were aggravated by the treatment.

The clinical and experimental data here presented have left us with the impression that injections of foreign protein are not likely to be of much value in the treatment of peptic ulcer except in occasional cases. The discomfort entailed did not justify the results obtained.

We have no exact information which would warrant our making guesses as to the mechanism which in some cases produces prompt relief of pain and discomfort.

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# THE METABOLISM OF LEUCOCYTES FROM NORMAL AND LEUKEMIC BLOOD

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Warburg's studies (9) on the metabolism of tumor cells have stimulated great interest in similar studies of the blood corpuscles. Grafe (1) has shown that leucocytes consume considerable amounts of oxygen. Although it had previously been known that they are capable of splitting sugar, it remained for Levene and Meyer (2) to demonstrate their ability to produce lactic acid from dextrose. Maclean and Weir (3), in comparing the glycolytic power of leucocytes and erythrocytes, found that the former have a glycolytic power several hundred times greater than the latter. Metabolic studies have been carried out with a view of determining the oxygen consumption and sugar destruction of leucocytes, and of mature as compared with that of immature white corpuscles. These studies have yielded contradictory results. Daland and Isaacs (4) as well as Glover, Daland and Schmutz (5) concluded that mature leucocytes consume greater amounts of oxygen than do immature white cells. The latter investigators (5) also concluded that the glycolytic function of the leucocytes, at least in whole blood, is inversely proportional to the maturity of the cells. These findings could not be confirmed by Barron and Harrop (6), who emphasized the depressing effect of concentration on both the oxygen consumption and sugar destruction of white blood cells.

The object of this paper is to study further, with improved technic, the effect of cell maturity and cell concentration on the metabolic activity of leucocytes.

## METHOD

Whole blood containing varying numbers of leucocytes from patients suffering from myeloid and lymphoid leukemia and from cases of leucocytosis due to various causes was studied. Arterial blood was collected in most instances. This was done to avoid the injury to the cells which results from manipulation incidental to aeration of venous blood. Samples were collected in purified heparin and immediately studied. Oxygen consumption was determined in Barcroft Warburg manometers at 37.5° C, the readings being taken at 5 minute intervals for 30 minutes. Sugar determinations were made immediately before placing the samples in the microrespirometers, and directly after the experiment. The samples to be studied were subjected to as little manipulation as possible and centrifuging was entirely avoided in view of its damaging effects on the respiration and to a lesser extent on the glycolytic activity of the white blood cells, as demonstrated by Fujita (7). Varying



TABLE I

Summary of the total leucocyte counts and differential values of all the cases studied

Blood maturity group	Number of observations	Total white blood cells	Total number of granulocytes	Total number of myeloblasts	Total number of myelocytes	Total number of juvenile forms	Total number of segmented forms
(a) Myeloid leukemia							
I	9	per c.mm. 6600-25 900	6140-24 600	232-630	595-1620	1150-4230	3760-20 800
II	19	17 925-269 000	17 400-262 000	599-13 450	2950-51 200	5920-94 200	8060-103 500
III	8	9325-49 600	8575-43 700	840-14 400	2800-5460	1040-3690	1305-21 800
(b) Myeloid reactions							
I	23	6500-35 000	5600-32 900	0	62-1750	560-6500	4670-25 900
(c) Lymphoid leukemia							
Blood maturity group	Number of observations	Total white blood cells	Total number of cells of lymphoid series	Total number of lymphoblasts	Total number of young lymphocytes	Total number of adult lymphocytes	
I	17	per c.mm. 17 500-706 000	16 800-696 000	105-2020	1015-33 700	15 700-696 000	
II	8	67 500-511 000	65 500-496 000	9250-70 000	23 900-181 000	32 300-245 000	

The complete data, including red cell count, reticulocyte count, degree of polychromatophilia, number of nucleated red blood cells, platelet count and oxygen consumption and glycolysis of the individual experiments are not published for the sake of brevity.



here be made that where such a relatively small number of observations is considered, too great stress must not be laid on the values obtained. The correlation in both cases is nonlinear. The ratio of correlation ( $\eta$ ) between  $K_1$  and leucocyte count is 7492 which becomes 7010 when corrected for number of arrays. The number of arrays used in the calcu

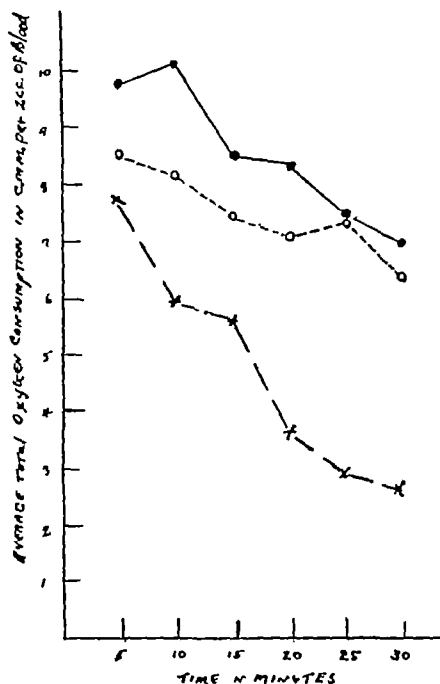


FIG 1 THE AMOUNT OF OXYGEN CONSUMED BY LEUCOCYTES FROM NORMAL AND LEUKEMIC BLOOD MEASURED AT 5 MINUTE INTERVALS AVERAGE OF ALL EXPERIMENTS

- — ● Myeloid Leukemia
- - - ○ Lymphoid Leukemia
- x - - - x Myeloid Reactions

lation of this constant was 10 and therefore  $(K - 1)/N$ , or the mean value of  $\eta$  where correlation is actually zero, is 1765. That the ratio of correlation between magnitude of leucocyte count and  $K_1$  is probably significant is shown by the fact that  $\eta$  differs from  $\sqrt{(K - 1)/N}$  ( $= 4202$ ) by more than  $17/\sqrt{N}$  ( $= 2380$ ), a criterion which has been set up by Pearson.





imately 30,000, following which a less rapid diminution was observed up to about 70,000 leucocytes. Beyond this point the depressing effect of concentration was so great that oxygen consumption was diminished relatively little in proportion to large differences in leucocyte counts.

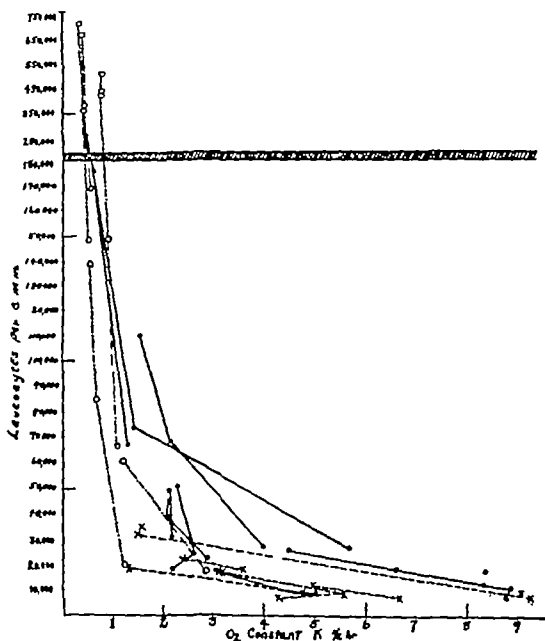


FIG 3 Relation of Number of Leucocytes to Oxygen Consumption Constant  $K_2$  (30 MINUTES)

- — ● Myeloid Leukemia
- - - - ○ Lymphoid Leukemia
- x - - x Myeloid Reactions

$$* K_2 = \frac{\text{c.mm O}_2 \text{ per 2 cc blood}}{\text{cell concentration (in thousands)} \times \text{time (30 minutes)}}$$

The correlation between  $K_2$  and the leucocyte count is greater than that between  $K_1$  and the number of white corpuscles. In other words the longer the experiment continues the greater is the depressing effect of the number of leucocytes on the oxygen consumption. This is apparent in comparing Figures 2 and 3 and is indicated by the greater value of the correlation ratio for  $K_2$  as compared with that for  $K_1$ . It is evident from

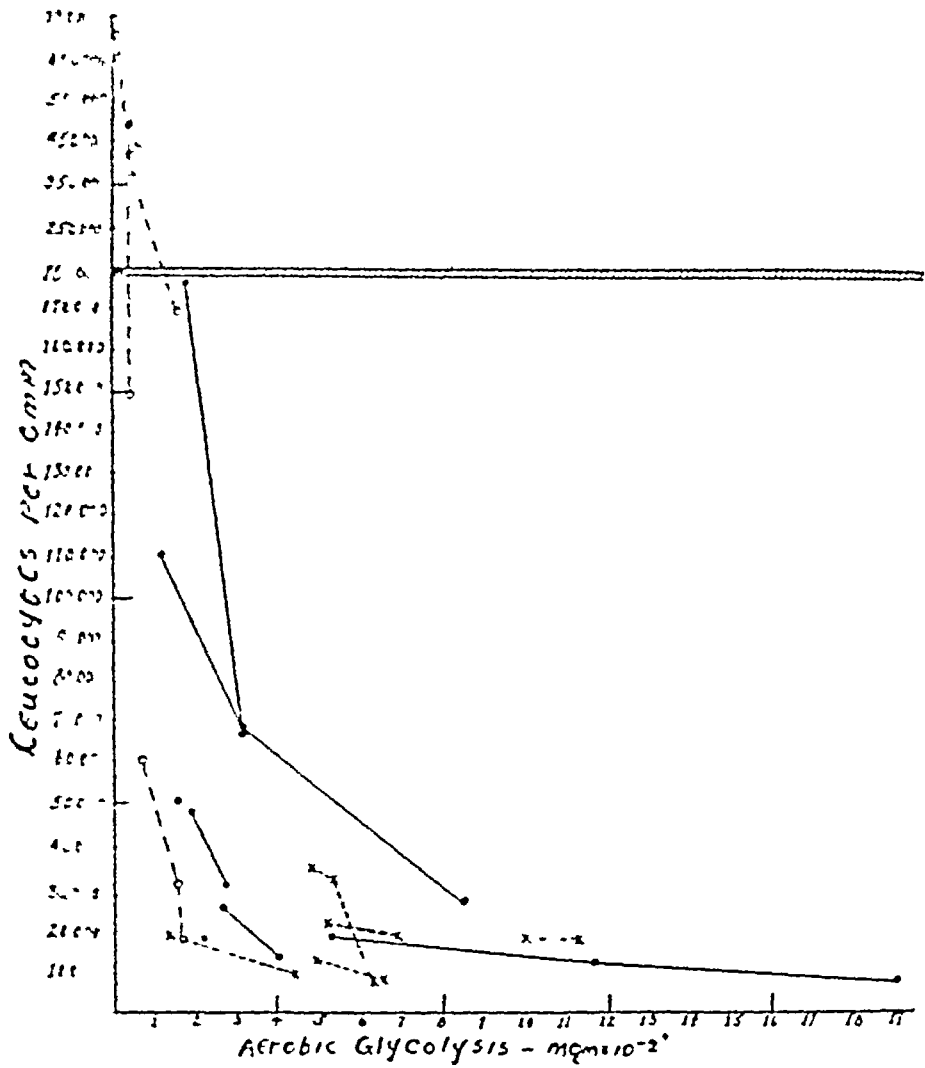


FIG. 4. RELATION OF NUMBER OF LEUCOCYTES TO AEROBIC GLYCOLYSIS—  
Mgm(× 10⁻²)

- — ● Myeloid Leukemia
- - - - ○ Lymphoid Leukemia
- × ..... × Myeloid Reactions

$$f = \frac{\text{mgm of sugar per hour per 2 cc blood}}{\text{cell concentration (in thousands)}}$$

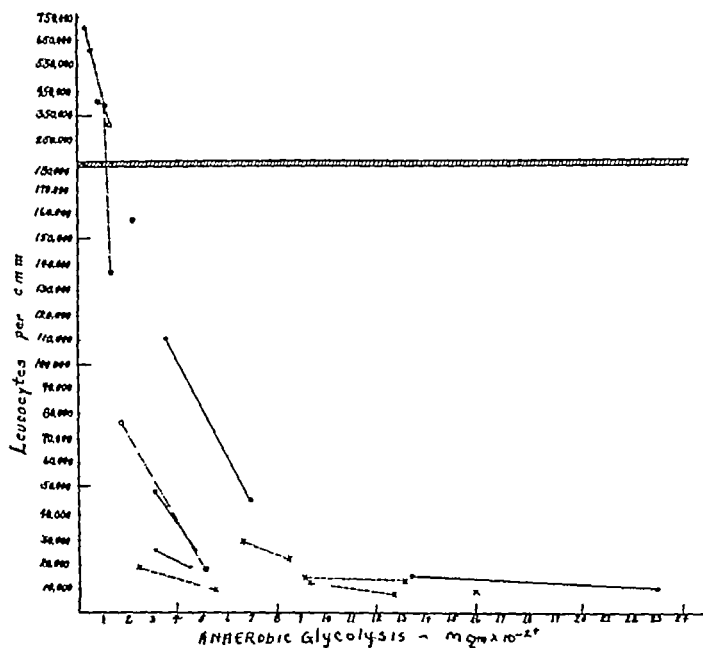


FIG 5 RELATION OF NUMBER OF LEUCOCYTES TO ANAEROBIC GLYCOLYSIS—  
Mgm( $\times 10^{-2}$ )

- — ● Myeloid Leukemia
- - - - ○ Lymphoid Leukemia
- x x Myeloid Reactions

$$\dagger K_G = \frac{\text{mgm of sugar per hour per 2 cc. blood}}{\text{cell concentration (in thousands)}}$$



TABLE III  
*Comparison of metabolism of leucocytes from cases of leucocytosis and myeloid leukemia and from myeloid as compared with lymphoid leukemia*

Type	O <sub>2</sub> consumption constant *				Aerobic glycolysis			Anaerobic glycolysis		
	Number of observations	Average white blood cell count	X <sub>1</sub> 10 min. unit period	X <sub>2</sub> 30 min. unit period	Number of observations	Average white blood cell count	Glycolysis† X 10 <sup>-3</sup>	Number of observations	Average white blood cell count	Glycolysis† X 10 <sup>-3</sup>
Chronic myeloid leukemia	10	19,363	6.02	5.51	6	per c.mm. 16,850	8.55	5	per c.mm. 18,495	9.75
Myeloid reactions	12	12,685	6.33	4.91	10	13,912	6.12	8	15,481	8.42
Chronic myeloid leukemia	7	117,142	1.71	1.40	5	93,500	2.07	2	135,100	2.84
Chronic lymphoid leukemia	6	113,916	1.21	0.83	3	129,333	0.85	2	106,700	1.47

\* c.mm O<sub>2</sub> per 2 cc blood

† cell concentration (in thousands) X time (hours)  
 † mgm sugar per hour per 2 cc. blood  
 † cell concentration (in thousands)



TABLE IV  
*Relationship of metabolism to maturity of leucocytes*

Maturity	Number of observations	Average leucocyte count	Oxygen consumption constant *		Maturity	Number of observations	Average leucocyte count	Glycolysis †	
Group number			K <sub>1</sub> 10 min utes	K <sub>2</sub> 30 min utes	Group number			Aerobic mgm. × 10 <sup>-3</sup>	Anaerobic mgm. × 10 <sup>-3</sup>

(a) *Myeloid series*

I	17	13 668	6 80	5.30	I	9	7 898	8 16	14 84
III	4	16 741	4 64	3 73	I	14	15 268	6 92	8 64
II	5	24 005	4 19	3 49	III	2	21 350		5 85
I	4	28,544	3.89	2 48	I	7	27 396	3 93	6 06
III	1	48 700	2 84	2 12					
II	9	100,567	1 91	1 55	II	1	27 500	8.51	
					III	2	30 700	2 73	
					III	2	49 100	1 84	3 02
					II	9	92 600	2 08	4 19

(b) *Lymphoid series*

I	2	18,812	2.23	2 05	I	2	24 000	1 57	5 02
II	2	106 250	1 82	1 02	I	2	68 800	0 66	1 67
I	4	109 000	0 91	0 75	II	2	143 500	0.38	1 27
I	3	298,300	0 46	0 45	I	3	285,700	0 96	1 13
II	3	462,700	0 75	0 77	II	2	393 000		0 93
I	2	684,500	0.36	0.38	II	3	462 700	0 41	
					I	4	666,000	0 08	0 42

\*  $\frac{\text{c.mm O}_2 \text{ consumed per 2 cc blood}}{\text{cell concentration (in thousands)} \times \text{time (hours)}}$

†  $\frac{\text{mgm sugar per hour per 2 cc. blood}}{\text{cell concentration (in thousands)}}$

respiration of the leucocytes. In Table V the cases have been arranged according to their erythrocyte counts. In a group of the myeloid cases of which the average red cell count was 2.8 million and the leucocyte count 12,109,  $K_1$  and  $K_2$  were 7.19 and 5.98 respectively, as contrasted to another series in which the red count averaged 4.6 million and the white cells 16,590, where  $K_1$  was 6.31 and  $K_2$ , 4.36. The differences in these oxygen constants were no greater than would be expected in view of the differences in the leucocyte counts. Essentially the same is true for the other groups tabulated. One must conclude that with our present methods of study any influence exercised by the red blood cells on the metabolism of leucocytes, if present, cannot be determined.



TABLE V  
Relation of red cell count to oxygen consumption constant

Range of red blood cell counts	Myeloid cases with white cell counts of 10,000 and less				All lymphoid cases				Myeloid cases with white cell counts over 10,000			
	Number of observations	Average		O <sub>2</sub> consumption constant*	Number of observations	Average		O <sub>2</sub> consumption constant*	Number of observations	Average		O <sub>2</sub> consumption constant*
		Red blood cells	White blood cells			Red blood cells	White blood cells			Red blood cells	White blood cells	
		millions per c mm	per c mm	As 10 min. utes		millions per c mm	per c mm	As 10 min. utes		millions per c mm	per c mm	As 10 min. utes
0.0 to 2.0	9	1.1	19,158	1.18	1	0.8	121,625	1.32	2	0.9	39,700	3.01
2.1 to 4.0	8	2.3	12,109	7.19	7	2.6	320,214	1.07	5	2.8	122,220	1.61
4.1 to 6.0	6	1.6	16,590	6.31	2	1.5	684,500	0.37	6	1.8	58,316	2.21

\* c mm, O<sub>2</sub> consumed per 2 cc blood  
cell concentration (in thousands) × time (hours)

## DISCUSSION

Our observations, as described in this paper, show definitely the important part that concentration plays in the "in vitro" metabolism of leucocytes. Their oxygen consumption and aerobic and anaerobic glycolysis are inversely proportional to the number of white blood cells present. The glycolytic function is not as markedly affected as is respiration, and this may perhaps be explained by the general observation that glycolysis is less easily influenced by external factors. Our results fail to substantiate the views advanced by Daland and Isaacs (4) and by Glover, Daland and Schmitz (5) concerning the influence of maturity of the cells on their metabolism. In fact, their results are more plausibly explained on the basis of concentration than on that of maturity. If the age of the leucocyte is a factor in determining its oxygen consumption and glycolysis, its influence is entirely obscured by the effect of concentration, and can hardly be determined with our present methods.

Warburg (9) has demonstrated the difference in metabolism of various types of tissues. Malignant tumors destroy large amounts of sugar aerobically and anaerobically while they consume moderate amounts of oxygen. In contrast to this type of metabolic activity normal adult tissues with the exception of retina have a comparatively small aerobic and anaerobic glycolysis with a high respiratory function. Embryonic tissues assume an intermediate rôle, their aerobic glycolysis being small while the anaerobic glycolysis is quite pronounced. The oxygen consumption of embryonic tissues is similar to that of normal adult tissues but higher than that of malignant tumors. According to Bakker (10) who worked with polynuclear and mononuclear cells obtained from sterile rabbit peritoneal exudates, these cells behave in their metabolism like cancer cells. Fujita (7) on the other hand, working with rats' blood found that the leucocytes have a metabolism similar to that of embryonic tissue. Fleischmann and Kubowitz (11) used goose and rabbit leucocytes suspended in Ringers' solution and found their metabolism to resemble that of malignant tissues.

Our results indicate that the granulocytes of both the leukemic and leucocytic group with their comparatively large aerobic and anaerobic glycolysis resemble malignant tissues in their metabolism, whereas lymphocytes from cases of lymphatic leukemia are similar in their metabolic activity to that of normal adult tissues.

## SUMMARY

Oxygen consumption and aerobic and anaerobic glycolysis of leucocytes have been studied in a series of cases of leucocytosis, and acute and chronic myeloid and lymphoid leukemia. In all 84 experiments were carried out, of which 23 were in cases of leucocytosis, 36 in myeloid leukemia and 25 in lymphoid leukemia. These represent a wide range of leucocyte counts and varying degrees of white cell maturity.

## CONCLUSIONS

1. The oxygen consumption and glycolytic activity of leucocytes from normal human blood is, *in vitro*, inversely proportional to the concentration of the white cells in the blood.

2. The amount of oxygen consumed rapidly decreases the longer the duration of the experiment. The maximum oxygen consumption per unit of time is reached during the first 10 minutes.

3. When allowance was made for the influence of concentration no differences in the metabolism of mature and immature leucocytes could be observed nor between that of leucocytes from normal as compared with leukemic blood.

4. The oxygen consumption of granulocytes is somewhat greater than that of lymphocytes, while the former have a glycolytic power about twice as great as the latter.

5. The number of red blood cells in the sample did not appear to influence the oxygen consumption of the leucocytes.

6. The metabolism of granulocytes under aerobic and anaerobic conditions resembles that of malignant tissues, while that of lymphocytes is similar to the metabolism of normal adult tissues.

We wish to acknowledge the valuable technical assistance of J. Walter Landsberg and Mrs. Florence White.

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# LIPID CONTENT OF TUMORS

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## INTRODUCTION

Studies bearing on the lipids of tumors have been made in most cases either with regard to the changes of the lipid content of the blood of tumor animals and patients or the relation between the lipids and the growth of the tumors

Of the lipids, cholesterol has been most frequently studied Roffo (1) reported that cholesterol is concerned with the development of experimental tumors and that an increase of cholesterol in blood plasma characterizes the so-called precancerous stage. Also the cells of malignant tumors cause considerable destruction of absorbed cholesterol Mattick and Buchwald (2) found hypercholesterolemia in the plasma of cancer patients with little change in the content of the corpuscles They found also an accumulation of total fatty acids in the plasma Kahn (3) showed that malignant tumors contained large amounts of cholesterol and its esters while the cholesterol content of the blood serum was lower than normal Bolaffi (4) could not find any particular change in the cholesterol content of the blood of tumor mice, but found an increase of cholesterol ester in the whole organism Burghelm (5) by histochemical examination found larger amounts of cholesterol in malignant tumors than in benign ones and also found a temporary increase of cholesterol in the blood of cancer patients after treatment by x rays He believed that this increase of cholesterol in the blood was caused by the destruction by the x rays of cells containing large amounts of cholesterol

Phosphorus of the blood of the tumor animals interested Haam (6), Enselman (7) and Roffo (8) who made studies of the phospholipids and nucleoproteins

The lipids of tumor tissue were first investigated by Bullock and Cramer (9) who found that they showed both quantitative and qualitative differences in different strains of tumors and that rapidly growing tumors contained more phospholipid and less cholesterol than the slowly growing ones

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Regarding the influence of the lipids on the growth of tumors little can be said despite a good many investigations. Maisin (10) stated in his recent work that diet can influence the appearance and evolution of tar cancers. Preceding closely the development of tar cancer and during its evolution the metabolism of fat is defective as shown by cholesterinemia. Feeding of liver lipids or ether extract of liver has no accelerating effect on the growth of the tumor, but rather an inhibiting one when compared with a raw beef muscle or a purely vegetarian diet. A diet of brain, rich in lipids, seems to have the same inhibiting action on tar cancer as liver lipids and to an even greater extent. Rondoni (11) injected cholesterol suspended in guinea pig serum and physiological sodium chloride solution into rats inoculated with tumors and found that it unquestionably promoted the growth of the tumor, whereas lecithin showed itself to be an inhibitor of the tumor growth. The acetone extract of tissues promoted growth. Friedberger and Simke (12) concluded that the growth of the tumor is dependent upon the quantity and quality of the diet. A deficient diet or any diet which causes malnutrition of the animals prevents the growth of tumors. Similar statements have been made by other investigators, Caspari and Ottensooser (13) and others.

The present communication is a preliminary report chiefly concerned with the lipid contents of various tumors with reference to their physiological or pathological significance.

#### LIPID CONTENT OF VARIOUS TUMORS

##### *Materials and methods*

The materials used were both human and animal tumors. The former were mostly obtained by surgical operation, a few at autopsy. The animal tumor was the Mouse Adenocarcinoma of Buffalo Strain 3. The tumors, as soon as possible after the removal, were cut up fine with scissors into a watch glass with a cover. The whole was weighed, then one part of the minced tissue was transferred to a mortar in which the tissue was ground with sand, while the other part of the tissue was used for the determination of water content. After grinding, the mass of tissue and sand in the mortar was transferred to an Erlenmeyer flask using a minimal amount of water (about 10 cc. was used to transfer about 3 grams of the ground tissue). The extraction of the lipids was first carried out with two portions of alcohol-ether mixture (3 parts alcohol and 1 part ether) for 10-15 minutes each, then with ether for 5 minutes with return condenser on the steam bath. The extracts were made so that about 1 gram of the moist tissue was contained in 100 cc. of the solvent.

The methods of lipid analysis were as follows: phospholipid, Bloor's oxidative method (14), total fatty acids, Bloor's oxidative method (15), cholesterol both colorimetric and oxidative methods, but later the oxidative method (16) only. Neutral fat was calculated by subtracting the

fatty acids of the phospholipid and cholesterol ester from the total fatty acids according to the following formula

$$\text{Fat F A} = \text{Total F A} - \text{Phospholipid F A} \left( \frac{2}{3} \text{ of the weight of phospholipid} \right) - \text{Cholesterol ester F A} \left( 43 \text{ per cent of the weight of ester} \right)$$

The "residual unsaponifiable substance" was calculated by subtraction of the total cholesterol from the total unsaponifiable substance which was determined by the following procedure

An aliquot of the lipid extract was saponified with 2 cc sodium ethylate (2.5 grams metallic sodium in 100 cc absolute alcohol) by heating on the steam bath almost to dryness, then acidified with dilute sulphuric acid and extracted with petroleum ether. The petroleum ether extract was transferred to a small separating funnel and to it was added the same amount of absolute alcoholic sodium ethylate (1 gram metallic sodium in 100 cc absolute alcohol). It was shaken well, then water was added to make the concentration of alcohol in the funnel about 50 per cent, the mixture shaken well again and left for a while. At this concentration of alcohol, the fatty acids set free by saponification enter the alcohol fraction while the unsaponifiable fraction remains in the petroleum ether which separates from the dilute alcohol. The alcohol fraction was washed with a small amount of petroleum ether again. Both of the petroleum ether fractions were combined in a glass stoppered Erlenmeyer flask, evaporated to dryness on the steam bath removing the last trace of the petroleum ether by a gentle current of the air and the lipid content determined by Bloor's oxidative method (15). The material was calculated as cholesterol using the factor 3.90. Objection may be made to the use of this factor for the calculation since the nature of the residual unsaponifiable substances is unknown. However for the purpose of this comparison the above calculation is allowable. The procedures for the separation are modifications of those of Kumagawa and Suto (18).

To test the recovery of unsaponifiable substance, cholesterol in this case, a lipid mixture containing known amounts of oleic acid and cholesterol was prepared and a separation made according to the directions above. It was found that the recovery was complete to within a small fraction as the following results show

*Total unsaponifiable substance*

Theoretical mgm.	Found mgm.	Difference per cent
1.85	1.87	+ 1
1.85	1.81	- 2
1.47	1.46	- 0.8
1.47	1.43	- 3
1.47	1.38	- 6

The results of the lipid analysis of various tumors are given in Table I

## DISCUSSION

The lipid content of various tumors is considerably different according to the nature of the tumors as shown in Table I. The high content of phospholipid in cancers, particularly in human cancers compared with other tumors is especially striking. The cholesterol content seems

TABLE I  
*Lipid content of tumors*  
(The content is expressed in grams per 100 grams dry tissue)

Tumors		Dry substance	Phospholipid	Cholesterol ‡		Fat	Residual unsaponifiable	
				Total	Free		Content	Per cent of total unsaponifiable
A		<i>per cent</i>						
Fibrosarcoma	1	19.59	5.720	2.107	1.585	2.100		
	2	13.13	4.733	1.700	1.690	3.471		
Neurofibroma	1	14.86	4.104	1.338	1.158	1.620		
Fibromyoma (uterus)	1	21.77	2.283	0.685	0.668	1.468		
	2	20.51	2.428	0.671	0.623	1.468		
	3	20.74	2.108	0.667	0.630	0.650	0.128	16.1
	4	21.41	2.448	0.722	0.680	0.200	0.333	31.6
Colloid adenoma (thyroid gland)	1	24.58	1.395	0.470	0.405	0.395	0.197	29.6
B								
Carcinoma (stomach) *	1	19.59	7.065	1.533	1.472	2.529		
Carcinoma (pancreas) *	1	25.40	9.573	0.781	0.413	27.444		
Carcinoma (breast)	1	20.21	4.462	1.258	1.200	8.300	1.300	51.0
	2	24.95	4.092	1.145	0.725	9.900	0.928	44.8
	3	18.17	3.900	1.193	0.971	13.400	0.989	45.0
Carcinoma (uterus)	1	18.16	6.195	4.677	1.435	1.790	1.293	21.7
Carcinoma (colon)	1	20.53	5.808	1.525	1.150	2.750	0.685	31.0
	2	17.05	6.016	1.992	1.465	1.770	3.088	
Uterus muscle †		20.33	3.508	1.015	1.000	0.675		
Colon tissue †		16.53	2.077	1.023	0.965	14.700		
C								
Mouse carcinoma	1	19.18	10.724	2.085	1.778	1.352	1.378	39.8
	2	18.93	6.308	1.891	1.591	5.861	0.872	31.5
	3	19.10	7.612	2.983	2.186	4.889	0.648	17.9
	4	17.88	6.570	2.362	1.590	5.600	0.730	23.6
	5	17.98	6.320	2.373	1.402	2.730	0.693	22.6
	6	19.83	5.190	2.495	1.710	4.090	1.325	34.7

A Human tumors other than cancer    B Human cancers    C Mouse cancers

\* Tumors obtained at autopsy

† Muscle of the uterus, mucosa and submucosa of the colon near the tumors which were analysed

‡ Cholesterol was determined by the oxidative method

higher in cancers than in the others, but not so high as the phospholipid. In two cases of human cancer, colon and uterus cancer, the lipid content of these tumors were compared with those of the mother tissues upon which the tumors were growing and it was found that the phospholipid content of these malignant tumors was much higher than that of the mother tissues. The residual unsaponifiable substances were not determined in all tumors due to the lack of material.

#### SUMMARY

It is difficult, strictly speaking, to classify tumors into malignant and benign ones. The transplantable mouse carcinoma may also be greatly different from the human carcinoma. However, the tumors of Group A may be classified as mostly benign, whereas Group B and probably C are no doubt malignant. If this classification be granted, the following can be stated as the summary of this experiment.

Malignant tumors contain a much higher percentage of fatty substances—neutral fat, phospholipid and cholesterol—than the less malignant ones, a high phospholipid content being especially characteristic of malignancy.

The writers wish to express their cordial thanks to Drs J J Morton and W J M Scott of the Surgical Department and to Dr K M Wilson of the Gynecological Department of this Medical School for providing materials of this experiment.

NOTE. Since this work was first reported (17) there have appeared two pieces of work on the same topic by LeMay (19) and Biench, Detzel and Lang (20). The results are similar to those reported above and so need not be discussed but taken together these investigations leave little room for doubt that malignant tumors are characterized by a much higher content of phospholipid and probably of cholesterol than benign tumors or than the corresponding normal tissues.

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# PLASMAPHERESIS EDEMA I THE RELATION OF REDUCTION OF SERUM PROTEINS TO EDEMA AND THE PATHOLOGICAL ANATOMY ACCOMPANYING PLASMAPHERESIS<sup>1</sup>

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Almost forty years ago, Starling (1) measured the osmotic pressure of serum protein and showed that exchanges of fluid between the blood plasma and interstitial spaces could be explained by the diminution of the capillary hydrostatic pressure of blood in passing from the arteries to the veins and the osmotic pressure of the proteins Krogh (2) has elaborated this idea and given more precise measurements of the factors governing the physiology of the capillaries

Clinical application of the physiological principles involving exchange of water across the capillaries has been surprisingly slow Starling's conception of the exchange of water between the blood and interstitial spaces would seem to have occurred to Bright (3) and some such idea was definitely proposed by Sabatier (4) and Solon (5) about a hundred years ago Although many writers seem to have appreciated the physiological rôle of serum proteins until the end of the nineteenth century, for a time their function was forgotten However, Epstein (6) seems to have been the first to emphasize that edema of nephritis and nephrosis could be explained by the low serum protein concentration Govaerts (7), Schade and Claussen (8), v Farkas (9) and others have made numerous measurements of the osmotic pressure of serum proteins and have shown that edema in renal disease is regularly accompanied by low serum protein concentration and low colloidal osmotic (oncotic) pressure, while in edema of heart disease not accompanied by proteinuria there is usually a high venous pressure

The association of edema with low serum protein concentration is now well established (Peters, Wakeman, Eisenman and Lee (10), Van Slyke et al (11) and others) Govaerts and v Farkas found that a one per cent solution of albumin exerts about three times as great an osmotic pressure as a one per cent solution of globulin This explains the fact that, in

<sup>1</sup> Part of the data in this paper was taken from the thesis of E B Hopper, done in partial fulfillment of the requirement for the degree of Doctor of Medicine in Yale University School of Medicine A preliminary report was read before the Philadelphia Pediatric Society, May 14 1929

edema due to low oncotic pressure, the albumin fraction of the plasma protein is usually low. At present it appears that albumin-deficit is the chief cause of the edema occurring in nephritis, nephrosis, nutritional edema and cachectic states.

Kohman (12) by feeding rats a diet low in protein, produced edema, which has been shown by Frisch, Mendel and Peters (13) to be accompanied by low concentration of serum protein. Shelburne and Egloff (14) have produced edema in a dog by feeding a low protein diet for a period of three months and then on observing a low serum protein concentration administering physiological saline by gavage. The type of experiments to be reported were first successfully carried out by Leiter (15) but similar work was started independently at about the same time by Barker and Kirk (16) and ourselves. Weech and Snelling (17) have recently performed similar experiments and will publish data showing the relation of the concentration of plasma proteins to chloride and sodium metabolism. The results of these groups of workers show that edema may be produced by reducing the content of plasma protein by repeatedly bleeding dogs, each time returning to the circulation the cells suspended in saline after removal of the plasma. The data of the various laboratories confirm and supplement each other and give a fair idea of the picture of experimental edema produced in such a fashion.

#### METHODS

Dogs were bled by puncture of the femoral artery, except in the first experiment. In this case, eleven bleedings were obtained from the jugular veins. The blood was collected in enough three per cent sodium citrate to give a final solution of about 0.3 per cent. The blood was centrifuged in 250 ml. centrifuge tubes and the plasma removed by suction. The centrifuge tubes were calibrated to permit rough but comparable estimations of the per cent by volume of red cells. The separated red cells, suspended in a 0.9 per cent solution of sodium chloride, were injected into the jugular veins. The succeeding bleeding was performed in most instances before returning the suspended cells, so as to increase the amount of plasma protein removed. In washing the cells into the veins, it was usually necessary to inject an amount of salt solution in excess of the amount of plasma removed. This excess is designated on the charts as extra I.V. saline. When the plasma proteins had reached a low level, physiological saline solution was given by gavage in order to have enough of this salt available to produce a large amount of interstitial fluid. Blood samples were withdrawn under liquid petrolatum in the morning before the first bleeding, that is, about eighteen hours after the previous bleeding or saline gavage.

The total protein was estimated in duplicate on one ml. of serum by the macro-Kjeldahl method, using 30 per cent hydrogen peroxide to

facilitate complete oxidation. Albumin was determined by the Howe (18) method of precipitating globulin with  $\text{Na}_2\text{SO}_4$  in the first two experiments and in the third experiment up to the eighth day. Thereafter Howe's method, using a mixture of  $\text{KH}_2\text{PO}_4$  and  $\text{K}_2\text{HPO}_4$  (1:2) to precipitate the globulin, was used in the majority of the determinations. The change in method was made because the globulin dropped so low in a rather unexpected manner, in Experiment III. Essentially identical results were obtained for three days in Experiment III and also on a number of specimens of human serum by both the sodium sulphate and the phosphate precipitation of globulin. In general, in our hands, the phosphate precipitation gives more uniform results from day to day on the same patient and does not give the occasional wide fluctuations that seem most likely to be due to errors in the method. However, in most cases the two methods give essentially the same results. In our opinion the methods of precipitation of globulin are all probably inaccurate and data involving globulin and albumin determinations must be used with caution. However, there seems to be good reason to believe that there are at least two fractions of serum proteins having quite different functions.

The present work is divided into two parts: (1) The relation of serum protein to edema and the anatomical changes produced by plasmapheresis, and (2) the chemical pattern of the blood produced by plasmapheresis. The first phase of the work is given in this paper and the second phase in the subsequent paper in this Journal.

The essential data are presented in the accompanying charts of Experiments I, II, III, IV, and V. The upper lines represent the concentrations of total protein, albumin and globulin in the serum. The lower lines represent the weights of the dogs and the total osmotic pressure of the serum proteins. These oncotic pressure values were calculated by multiplying the per cent of albumin by 5.6 and the per cent of globulin by 1.45. These are the factors given by Govaerts (7) for the conversion of concentrations of albumin and globulin to the osmotic pressure which they exert. The figures below represent the volume of the bleedings, the amount of 0.9 per cent saline which was injected above the amount necessary to replace the plasma removed, and the amount of 0.9 per cent salt solution given by gavage. Although corrected for the volume of citrate, the figures for the per cent by volume of erythrocytes are only approximate. At the top of the charts the amount of edema is represented by + — when the skin in dependent parts felt rather doughy, but there was no definitely pitting edema, + when there was pitting edema, and ++ when there were pitting edema and definite signs of ascites. At the end of the paper additional observations are recorded concerning each experiment. These notes are not complete protocols, but, together with the charts, report the facts that seem significant.

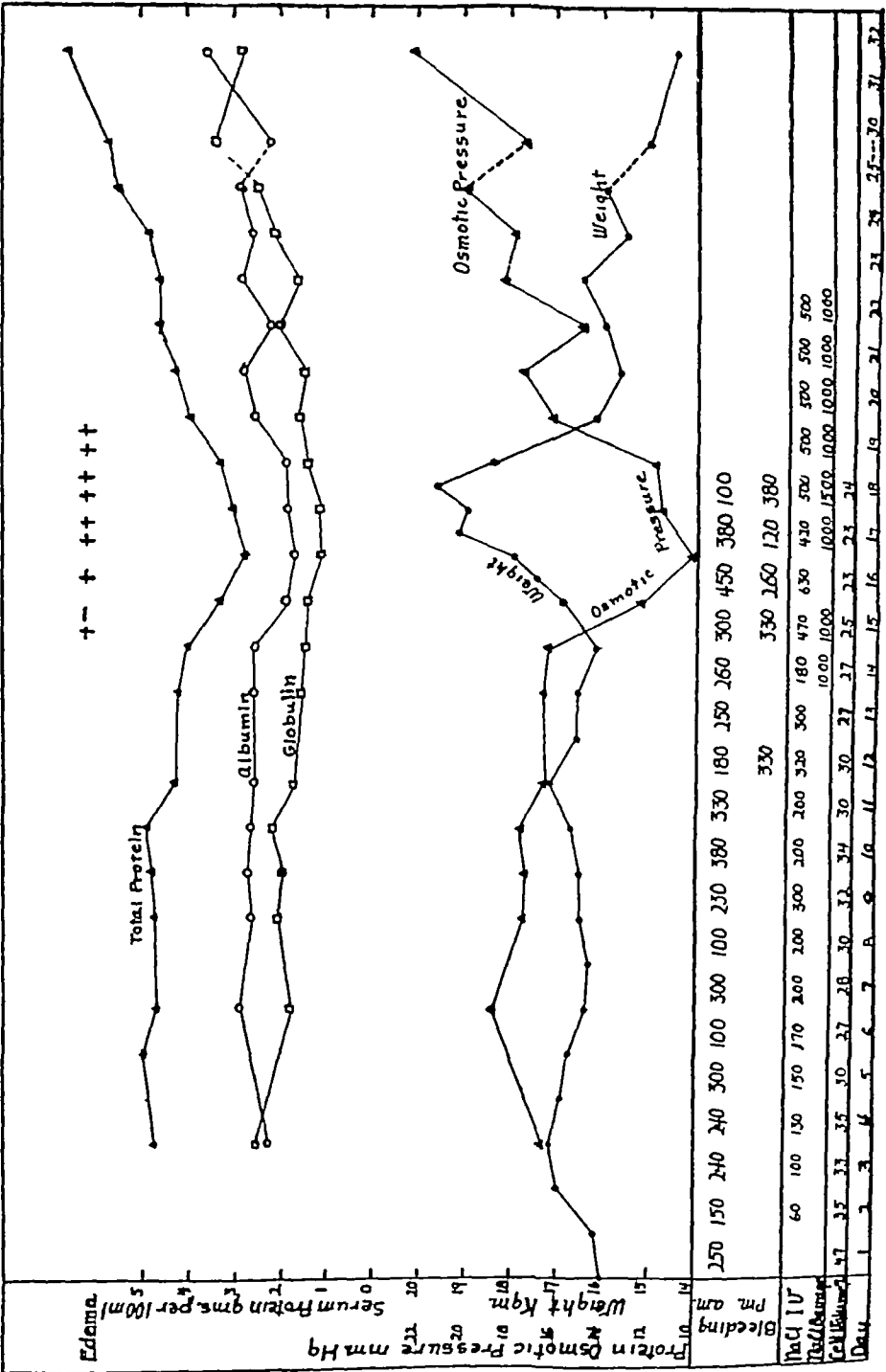


CHART 1 EXPERIMENT I

One would like to know the level of protein which is necessary for the production of edema. This can not be determined precisely from the data of these experiments. After a given bleeding and transfusion of cells suspended in saline solution, the plasma protein content was very low. However, the content rose fairly rapidly and reached the level

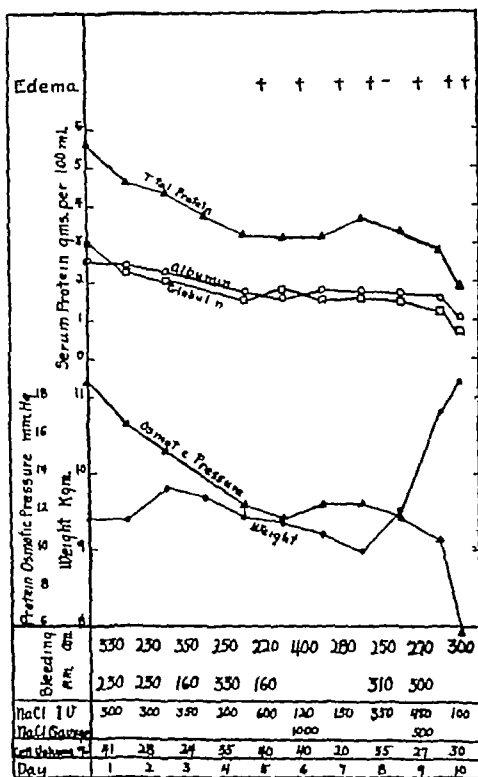


CHART 2 EXPERIMENT II

reported about eighteen hours later. Since the direction of water exchange is the best measure of the tendency to the formation of edema, an increase in weight is probably the best available evidence that the conditions necessary for edema production were present. However, at the time of taking the blood samples, the proteins may have reached already a level which would ultimately lead to a disappearance of edema.

Therefore, the morning level of protein that was accompanied by increase in weight and edema represents a figure probably somewhat higher than that which would be found if one could devise experiments which would lead to a constant low level of plasma protein

Bearing in mind the above considerations, the data are fairly consistent. Edema tended to appear when the total protein concentration fell below 3.5 to 3.0 per cent. As one might expect from the fact that a given

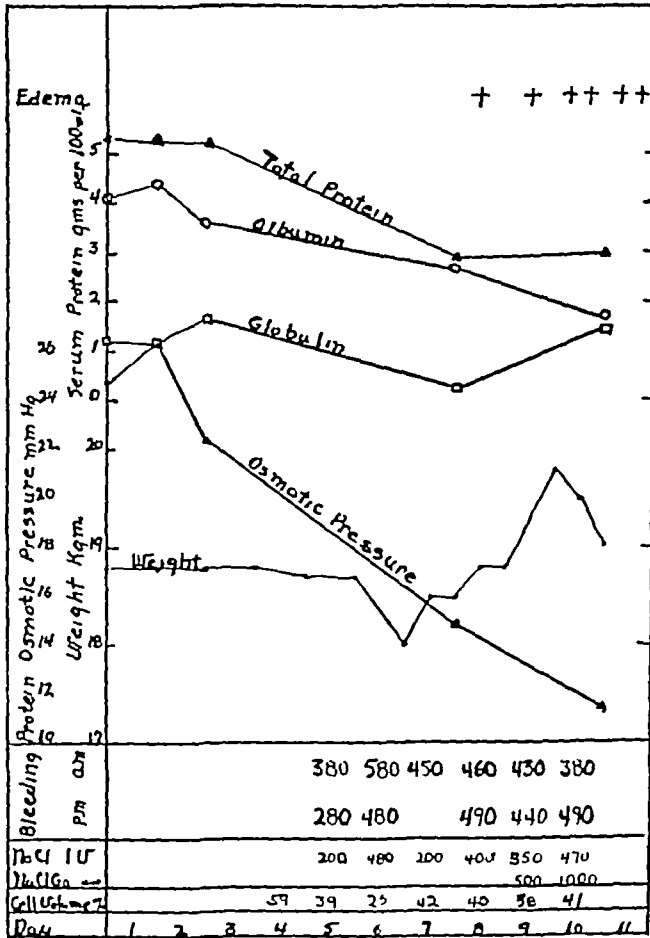


CHART 3 EXPERIMENT III

concentration of albumin has about three to four times as great osmotic pressure as the same concentration of globulin, the occurrence of edema is more closely related to the concentration of albumin than to the concentration of total protein. Thus edema occurred in all cases when the serum albumin concentration was below two per cent and was increasing when the albumin concentration was lower than 1.5 per cent. As the concentration of the serum albumin approached two per cent there was either questionable edema or a tendency to retention of water, as exhibited

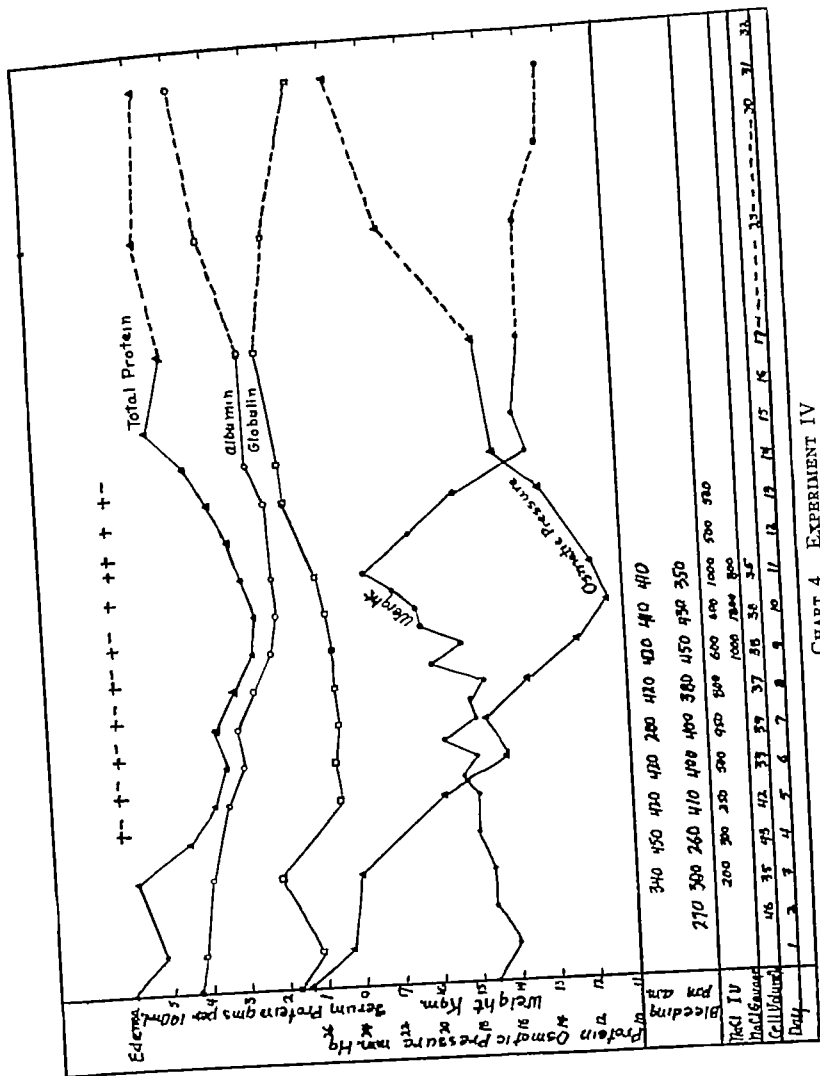


CHART 4 EXPERIMENT IV



by an increase in weight in the afternoon. The dogs were weighed morning and afternoon but the afternoon weight is not recorded on the charts except where it differed significantly from the morning weight. Experiment IV illustrates well the greater importance of albumin. In this case the total protein concentration was below 3.0 per cent for two days and

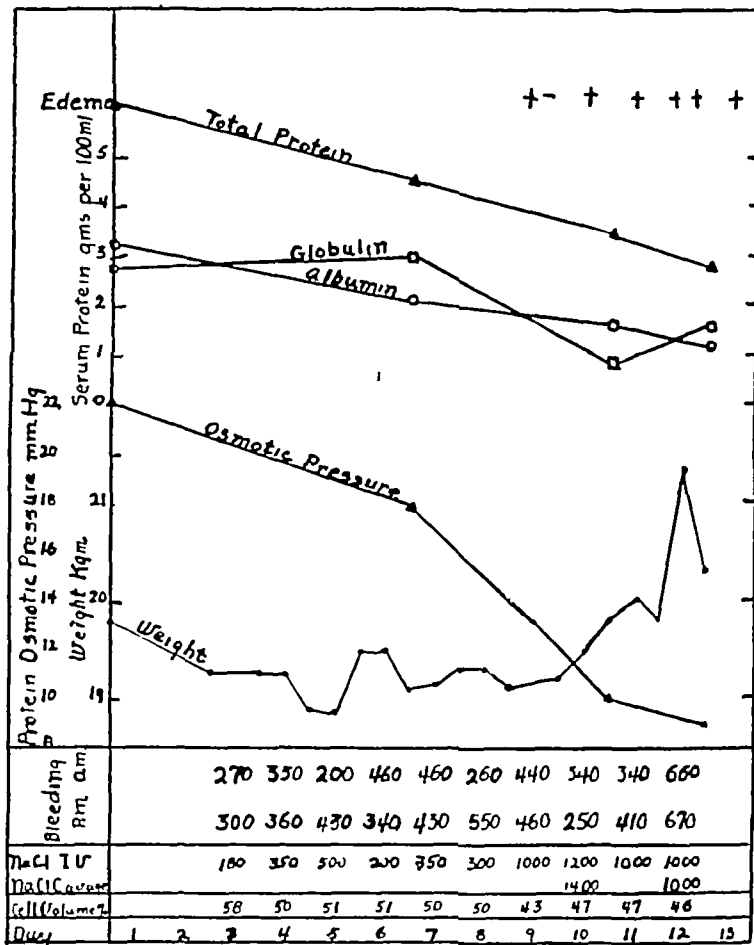


CHART 5 EXPERIMENT V

below 4.0 per cent for five days, but edema did not appear until the albumin had reached the critical level of 1.8 per cent.

The occurrence of edema should be more closely related to the total osmotic pressure of albumin plus globulin than to the concentration of albumin or of total protein. The line representing the osmotic pressure of the proteins demonstrates that this is the case. Edema seems to occur regularly when the osmotic pressure of the proteins drops below 11 to 12 mm. of mercury.

Presumably the large amounts of 0.9 per cent saline given by gavage and injected intravenously aggravated the edema. Clinically it is

recognized that NaCl is not the cause of edema but merely provides the chief ions of interstitial fluid and hence allows the body to increase the amount of interstitial fluid without greatly disturbing the electrolyte equilibrium existing between cellular and extracellular fluid. In Experiment I a liter of 0.9 per cent saline was given by gavage and 500 ml. was injected intravenously on each of the four days following the interruption of the bleeding. Nevertheless, the edema decreased rapidly. In Experiment IV, the intravenous injection of saline was continued two days after the bleeding and again the edema disappeared promptly. These experiments seem to prove that NaCl did not play a primary rôle in the production of the experimental edema.

The plasmapheresis reduced the albumin and globulin in about the same proportions. This fact may be appreciated best by examining Charts 6, 7 and 8. In these charts, the serum protein, serum albumin and serum globulin are charted as per cent of their values before bleeding was started. In Experiments I, II and V the concentrations of total protein, albumin and globulin, were all reduced in the same proportions while the bleeding was being continued. In Experiment IV the globulin seems to have been reduced a little more rapidly than the albumin. This result may be due in part to difficulty in the precipitation of the globulin in the two specimens taken before the bleeding. An average of the two values was taken in constructing the charts, but if the lower value had been used, the total protein, albumin and globulin lines would have been more nearly parallel. In any case, the globulin was very low during the period of rapid depletion of the plasma proteins and a small error in the determination of total protein and albumin would have a relatively large effect on the globulin, since this latter is determined by difference. For this reason, Experiment IV, in conjunction with the other experiments, seems to indicate that the total protein, albumin and globulin are reduced in about the same proportions during rapid plasmapheresis.

In Experiments I and IV it is clear that after the bleeding is stopped the globulin recovers its previous concentration more rapidly than albumin. After about a week the globulin is normal or above normal. The albumin does not reach its normal concentration until two to three weeks after the plasmapheresis has been discontinued. If the globulin goes above the normal value, it tends to return to the normal value at about the same time as does the albumin.

Barker and Kirk (16) report a more rapid reduction of albumin than globulin. Indeed in some of their experiments the globulin was above the normal level at the time edema appeared. They found the albumin level for the production of edema to be one per cent. A similar critical level for human subjects is indicated by a small number of cases reported in their paper.

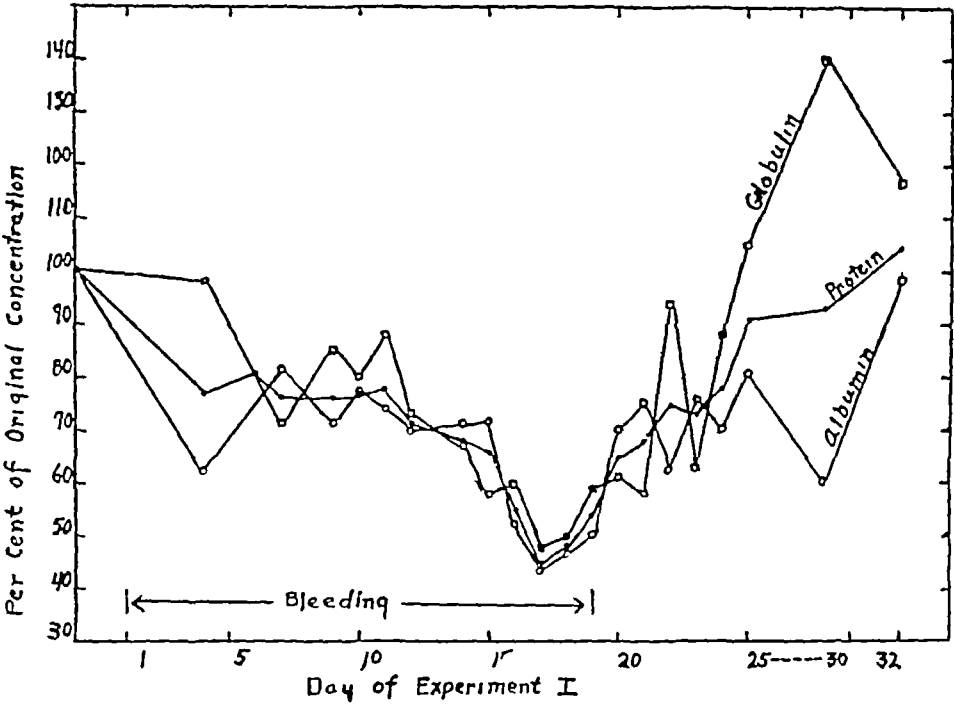


CHART 6

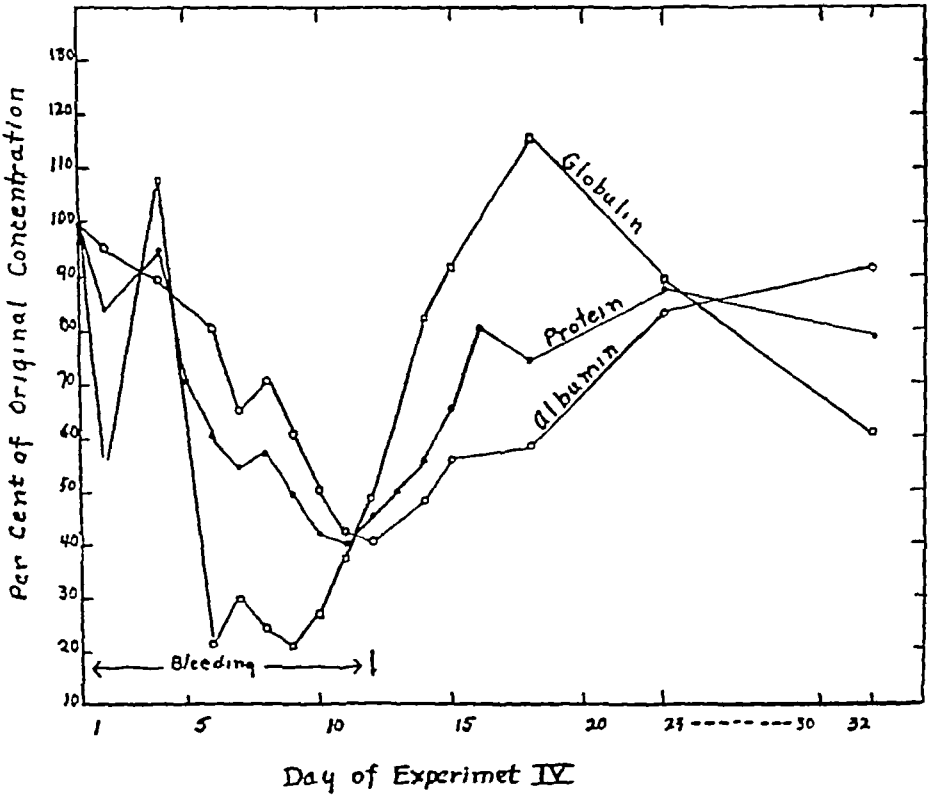


CHART 7

The discrepancy between their results and ours is apparently due to some difference in method in the precipitation of globulin. Both before and after bleeding, their globulin concentrations are higher than ours. The same may be said of their values for globulin in patients with edema. However, it should be pointed out that since the dogs studied by Barker and Kirk were bled over a longer period of time, they might have had a greater opportunity to develop a change in the relative concentrations of albumin and globulin, due to the difference in the rate of regeneration of the two protein fractions. In the experiments reported by them there seems to be a more rapid reproduction of globulin, which might explain

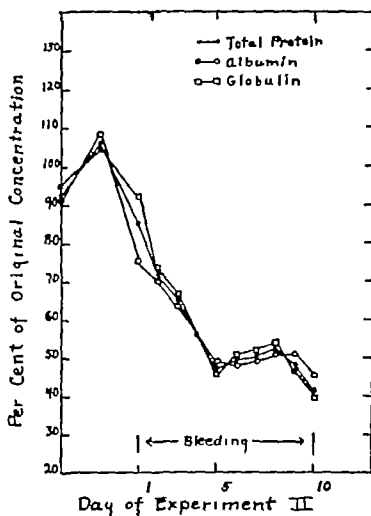


CHART 8

in part the relatively higher globulin concentration at the time of appearance of edema. Furthermore, Leiter (15) shows that globulin returns to the circulation more rapidly than albumin. Our figure for the critical level of albumin is in agreement with that of Leiter (15) and Weech and Snelling (17), it is somewhat lower, as one would expect, in dogs than in man, but is more consistent with the level found in other laboratories for human beings. Peters, Wakeman, Eisenman and Lee (10) found the critical level for albumin to be two per cent, while Van Slyke and many others (11) obtained a figure of 2 to 2.5 per cent.

*Pathological anatomy of dogs rendered edematous by  
plasmapheresis*

Barker and Kirk (16) and Barker (19) examined the kidneys of dogs with low concentration of serum proteins resulting from plasmapheresis. In those dogs killed in about two weeks, the kidneys were swollen and microscopically showed marked cloudy swelling, some desquamation of the epithelium and extrusion of cellular nuclei. After about two months, the kidneys were normal in gross appearance, but atrophy of the tubular epithelium was evident microscopically. "At six months the gross changes were pronounced. The capsule stripped easily but left a roughened and dimpled surface. The cortex was greatly narrowed and appeared marked with greyish-white streaks. Microscopic examination revealed marked scar-tissue replacement in the inner half, producing dimpling. There was great increase in the tubular degeneration, fatty infiltration and glomerular atrophy over that seen earlier in the process." Barker and Kirk thought the low concentration of the plasma proteins might be the effective cause of tubular degeneration and even glomerular atrophy, when long continued.

Leiter (15) examined a number of kidneys of dogs subjected to plasmapheresis. He was unable to substantiate Barker and Kirk. Such lesions as he saw were thought to be a spontaneous nephritis which occasionally occurs in dogs.

In the present study, one dog was killed after ten days of plasmapheresis, another after six days, and a third one after ten days. The last dog was used in both Experiments IV and V but almost a year elapsed between the two studies and the dog was in excellent condition before the second period of plasmapheresis.

We find considerable difficulty in interpreting our own histological findings. Grossly the organs appear normal. The sections of the kidneys show no scarring nor cellular infiltration. The glomeruli are normal. The convoluted tubules do not show sharp cellular outlines, the cytoplasm stains pink with hematoxylin and eosin and is rather coarsely granular. The nuclei appear essentially normal. In places the lumina of the tubules are filled with pink staining granular material. The picture is that of rather marked cloudy swelling. Such a change is frequent at autopsies on human beings and little significance is attached to the finding. However, the fact that the dogs were killed and the tissue immediately fixed with Zenker's fluid, makes one hesitate to disregard the cloudy swelling entirely.

The liver capillaries are dilated and the liver cells appear smaller than normal. There is some granular detritus between the liver cells and capillaries. The appearance approximates that described by Wolbach and Blackfan (20) in cases of nephrosis. The sections of the heart, adrenals and spleen show no significant changes.

The histological picture was controlled by examining two dogs subjected to the same procedures except plasmapheresis. In one dog, weighing 9 kgm, 500 ml of 0.9 per cent NaCl were injected intravenously and 700 ml of 0.9 per cent NaCl given by gavage for four days. On the fifth day one liter of salt solution was given intravenously and by gavage. On the sixth day the dog was killed with chloroform and the organs examined. Nothing abnormal was found grossly. The kidneys showed cloudy swelling just as pronounced as that of the dogs subjected to plasmapheresis. The liver cells were loaded with fat, but otherwise normal. The other tissues appeared normal microscopically. The other dog showed similar findings. The authors feel that any changes noted in the kidneys of the dogs subjected to plasmapheresis may be due to the large amounts of saline injected intravenously and given by gavage.

The changes in the kidneys are of a nature generally considered as repairable and, even if they are in part dependent on low concentration of serum protein, should not lead to permanent renal damage. Indeed, even in cases of "pure nephrosis," the kidneys show, for the most part, only a very marked cloudy swelling and some fatty changes in the tubules. Wolbach and Blackfan (20) felt that the lesions they found in the tubules were capable of repair in all but the most extreme instances. There seems to be little justification for assuming that low concentration of plasma protein is a cause of permanent renal injury in nephrosis or hydropigenous nephritis.

On several occasions urine was examined for albumin, sugar, casts and cells. Nothing suggesting renal injury was found. At the autopsies in Experiments II and III, the urine contained almost fifteen grams per liter of chloride expressed as NaCl. This finding indicates excellent ability to concentrate chlorides. Barker and Kirk (16) report occasional proteinuria and cylindruria. This occurred after several weeks of bleeding and was transient.

The analogy between the state of the dogs with low plasma protein concentration and cases of nephrosis, nutritional edema and cachectic states with edema is fairly close. In all, the production of edema is mainly dependent on low concentration of plasma albumin. In all, the edema fluid is practically free of protein. In all, symptoms of under-nutrition appear. Barker and Kirk found a low basal metabolic rate, which is frequently encountered in nephrosis as well as in starvation edema. We were impressed with the loss of weight accompanying plasmapheresis when there was no edema, as were Leiter (15) and Barker and Kirk (16). All the dogs tended to have loose stools during the edematous phase and in Experiments I and IV, this amounted to a moderate diarrhea. In Experiment I, the diarrhea occurred at a time when salt solution was being given by gavage, but in Experiment IV, the most marked diarrhea occurred on the thirteenth day when salt

solution had not been given by mouth for forty-eight hours. The loose stools would, therefore, seem to be part of the mechanism for getting rid of the large amounts of interstitial fluids during the period of recovery from edema. Similar observations have been fairly frequent in those cases of nephrosis which we have seen.

In the succeeding paper it will be pointed out that the chloride concentration of the serum tends to be high, as is also the case in clinical instances of edema with low concentration of serum protein.

#### SUMMARY

(1) Five dogs were bled and the cells reinjected after removal of the plasma proteins. By this procedure the albumin and globulin were reduced in approximately the same proportions. After the bleeding was stopped the restoration of globulin was more rapid than that of albumin.

(2) There seemed to be a critical concentration of 3 to 3.5 per cent total plasma protein at which edema appeared. The critical concentration of 1.5 per cent albumin seems to determine the production of edema more regularly than the total protein concentration. The occurrence of edema is most closely associated with a total protein osmotic pressure below 12 mm. of mercury, when the protein osmotic pressure is calculated according to Govaerts (7).

(3) Microscopic examination of the tissues of three dogs killed during the edematous phase revealed marked cloudy swelling of the tubular epithelium of the kidneys, but no lesions that did not seem readily reparable. The liver showed rather marked dilation of the capillaries and the liver cells were small and rather granular. Sections from heart, adrenal and spleen revealed no lesions. Similar cloudy swelling of the kidneys occurred in dogs subjected to the intravenous injection of saline and the administration of salt solution by gavage. In our opinion a low concentration of plasma protein is not a significant cause of permanent renal damage.

#### CONCLUSIONS

Dogs rendered edematous by plasmapheresis show a type of edema closely analogous to that of cases of nephrosis, hydropigenous nephritis, nutritional edema and cachectic states. Low plasma protein should not be considered a cause of permanent renal damage.

#### PROTOCOLS

*Experiment I.* Healthy adult male Airedale. The first eleven bleedings were obtained from jugular veins. Thereafter all bleedings were from femoral arteries. On the following days blood was lost: first day about 150 ml. through clotting, twelfth day about 100 ml. through clotting, and fifteenth day about 125 ml. through breaking of a centrifuge tube. The dog's appetite was poor from the tenth to the twentieth day of the experiment. During the period of

marked edema the dog seemed fairly well but was listless. There was evidence of weakness and nausea after the larger bleedings of the fifteenth and sixteenth days. The stools were loose during the edematous period and there was moderate diarrhea on the twenty first day. The appetite returned quickly when the bleeding was stopped. The dog lost body tissue during the experiment, as was evidenced by his appearance and weight after recovery.

*Experiment II* Rather small, healthy mongrel collie. All bleedings were from femoral arteries and transfusions into jugular veins. An attempt was made to feed the dog a diet high in fat and carbohydrate and low in protein, made up of a known mixture of casein, lard, dextrine, salts and vitamins. This led to visible lipemia during the first days but after the fourth day the dog refused to eat and the lipemia disappeared. He was then given the kennel diet of dog biscuit and a little meat, of which he likewise refused the greater part. About 150 ml of blood was lost through clotting on the first day. This dog showed considerable weakness and nausea and his veins seemed collapsed after each bleeding. On the fifth day he was transfused with about 80 ml of compatible erythrocytes from another dog. On the fifth and sixth days the transfusions were given before, rather than after, the bleeding. On the tenth day the transfusion was thought to be going into the lower part of the jugular vein but it was discovered later that most of it had infiltrated the neck and mediastinal tissues. The dog remained weak and had difficult, rather rapid deep breathing which suggested acidosis. Although he seemed to be recovering at 8 P. M. he was so uncomfortable that he was killed with chloroform after the removal of clear abdominal fluid and samples of arterial and venous blood.

At this time there was marked pitting edema of the chest walls and legs and shifting dullness in the abdomen.

*Postmortem urine*—Acid reaction. No reaction for protein or sugar. Microscopically no casts, erythrocytes or leukocytes. Chloride 14.95 grams NaCl per liter.

*Autopsy*—The abdomen contained about 500 ml of a slightly cloudy, colorless fluid. Together the chest cavities contained about 750 ml of similar fluid which contained some red cells which had apparently come from the mediastinum. The mediastinum and lower tissues of the neck were infiltrated with blood from the misdirected transfusion. The trachea may have been compressed by this fluid. Viscera were normal grossly.

Microscopic sections of the liver, spleen and heart were essentially normal, except for slight cloudy swelling. The kidney sections are discussed elsewhere.

*Experiment III* Large, fat Airedale rather old in appearance. All bleedings were from femoral arteries and transfusions into jugular veins. This dog seemed to be more affected by the bleedings than the other dogs. After the bleedings he usually exhibited nausea, spasmodic movements of the abdominal muscles and diaphragm. He vomited on the sixth, ninth and tenth days. On the eleventh day, having eaten little since the seventh, he was listless, but otherwise he acted fairly normally. He showed pitting edema in the loose skin around the Achilles tendon and the subcutaneous tissue elsewhere felt boggy. Arterial and venous samples were obtained and the dog was then killed with chloroform and abdominal fluid obtained after opening the peritoneum. Postmortem urine contained 14.8 grams Cl as NaCl per liter. The urine gave no reaction for protein or sugar and showed no abnormality microscopically.

*Autopsy*—Nothing remarkable was found grossly, except edematous subcutaneous tissues, about 400 ml of slightly cloudy, colorless abdominal fluid.



and about 75 ml of similar fluid in each chest cavity. Microscopic sections of the heart, lungs, spleen and liver were essentially normal except for slight cloudy swelling. The kidney sections are discussed elsewhere.

*Experiment IV* Healthy female German shepherd. All bleedings were from femoral arteries and transfusions into jugular veins. All samples were taken before bleeding in the morning. On the fourth day the dog vomited after each bleeding. The dog showed weakness and nausea after several of the larger bleedings, but seemed to feel well after being transfused. From the sixth to the twelfth day the appetite was poor. The stools were loose during the edematous phase. There was moderate diarrhea on the thirteenth day when the edema was clearing. On the twelfth day this dog was more edematous than any of the other animals. She showed marked pitting edema of the back, chest walls, front and hind legs. There was bulging of the flanks, shifting dullness in the abdomen, and considerable dyspnea, apparently due to chest fluid. The dog was somewhat apathetic and was not interested in food, but otherwise did not seem ill. She was lively the next day and apparently normal in two days. However, she became very thin during the next two weeks.

*Experiment V* Healthy female German shepherd. This was the same dog used in Experiment IV. Almost a year had elapsed since Experiment IV and she was in better physical condition for the second than the first experiment. All bleedings were from femoral arteries and transfusions into jugular veins. Bleedings caused little or no symptoms. At 6 P M of the twelfth day, definite pitting edema of the hind legs and abdominal wall and shifting dullness of the flanks were found. The next morning the shifting dullness of the abdomen was gone, but there was still moderate edema of the abdominal wall and hind legs. Arterial and venous samples of blood were taken and several unsuccessful attempts were made to obtain abdominal fluid. The dog was killed with chloroform.

*Autopsy*—Nothing abnormal was found grossly except edema of the subcutaneous tissues and about 50 ml of slightly blood tinged fluid in the abdominal cavity. The blood undoubtedly came from the attempts to obtain abdominal fluid just before killing the animal. Microscopic sections of the heart, spleen, liver, adrenals and kidneys were essentially negative, except for slight cloudy swelling of the liver and kidneys. The kidney sections have already been discussed in detail.

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## PLASMAPHERESIS EDEMA II THE EFFECT OF REDUCTION OF SERUM PROTEIN ON THE ELECTROLYTE PATTERN AND CALCIUM CONCENTRATION

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In a previous paper (1) it was shown that, when the concentration of serum protein had been reduced to about three per cent by plasmapheresis, dogs became edematous. The type of edema produced in this way is probably most closely analogous to that of nutritional edema.

In connection with the work on dogs reported in that paper, certain studies of serum and ascitic electrolytes were undertaken and will be discussed in this paper. Physiological saline was injected into two additional dogs to determine the effect of this procedure on serum electrolytes under conditions similar to those of the plasmapheresis experiments.

### METHODS

About eighteen hours after previous bleedings or administration of salt solution, blood was withdrawn from a femoral artery or jugular vein and was allowed to clot under oil. Serum was used for all analyses. Ascitic fluid was taken at necropsy in Experiments III and V but in the other cases by abdominal paracentesis. The following chemical methods were used: Bicarbonate, manometric method of Van Slyke and Neill (2) using 0.2 ml. chloride, Van Slyke (3) using potassium permanganate to oxidize the proteins; phosphate, Benedict and Theis (4); total base, Stadie and Ross's (5) modification of the Fiske method without removing phosphate or applying a correction for phosphate nitrogen by gross Kjeldahl on 1 ml. of serum using thirty per cent hydrogen peroxide to facilitate complete oxidation; nonprotein nitrogen, Folin and Wu (6); and calcium, Clark and Collip's (7) modification of Kramer and Tisdall's method. Albumin was determined by the Howe (8) method of precipitating globulin with  $\text{Na}_2\text{SO}_4$  in the first two experiments and in the third experiment up to the eighth day. Thereafter Howe's method using a mixture of  $\text{KH}_2\text{PO}_4$  and  $\text{K}_2\text{HPO}_4$  (1:2) to precipitate the globulin was adopted. The two methods give essentially identical results but we have found the phosphate method somewhat more consistent from day to day. Total nitrogen minus nonprotein nitrogen was multiplied by 6.25 to obtain protein. All determinations except phosphate and nonprotein nitrogen were run in duplicate.

All analyses are expressed in milli-equivalents (m eq.) of univalent base per liter of serum. In converting protein to equivalents of base, the factors of Van Slyke, Hastings, Hiller and Sendroy (9) were used: namely, albumin per cent  $\times 2.73$  and globulin per cent  $\times 1.87$ . Total carbon dioxide was converted to bicarbonate at pH 7.38 by multiplying carbon dioxide volumes per

cent by 0.423. Dibasic and monobasic phosphate were assumed to occur in the ratio of 4 : 1.

The values for concentrations in m eq per liter were converted to m eq per kilogram of water in calculating the data represented in Chart 2. The following formula of Van Slyke, Wu and McLean (10) was used for this purpose:  $\text{water} = 99 - 0.8 P$ , in which  $P$  is the protein in per cent. This formula has been tested a number of times and found to agree satisfactorily with the dried weight except in plasma having a large amount of lipid. It should be pointed out that although dried weight is not the same as water content, the error of such an assumption is probably small.

The experiments I, II, III, IV and V consisted of bleeding once or twice a day, centrifuging the citrated blood and returning the erythrocytes suspended in 0.9 per cent solution of NaCl after first removing the plasma. In order to facilitate the production of a considerable amount of edema, 0.9 per cent NaCl solution was given by gavage when the serum proteins had reached the level at which edema might be expected. The experiments should affect the electrolyte pattern of the blood (1) by reduction of the plasma protein concentration, (2) because of the administration of considerable quantities of NaCl, (3) through loss of blood and (4) perhaps by undefinable effects of the various experimental procedures. The hematocrit figures reported in the previous paper show that loss of erythrocytes should not effect the electrolyte pattern to any considerable extent. The reduction of hematocrit was from about thirty-five to twenty-five per cent. It was felt that the elapse of eighteen hours between the administration of salt solution and obtaining the sample of blood would allow any disturbance due to the NaCl solution to be adjusted so that the blood samples would represent essentially the effects of reduction of plasma protein. Nevertheless, as a control, two dogs were subjected to daily intravenous injections of 0.9 per cent NaCl and specimens removed in the morning. This duplicated the procedure of the previous experiments except for the plasmapheresis. The amount of saline injection in the control dogs is greater than that injected in the plasmapheresis experiments, but considerably less than the amount of saline given by gavage.

The data will be presented from three points of view: (1) the effect of change in serum protein concentration on the electrolyte pattern of the serum, (2) a comparison of the concentrations of ascitic fluid electrolytes with those of arterial and venous serum, (3) the effect of change in protein concentration on calcium concentration.

### *I The effect of change in serum protein on serum electrolytes*

Table I presents the data of the plasmapheresis experiments in detail. The following specimens are distinctly aberrant: the serum and ascitic fluids in Experiment II on the tenth day, which were taken about six hours after injection of physiological salt solution and which showed

TABLE I  
Electrolyte concentration of blood serum and ascitic fluid

Experiment number	Day	Sample*	Edema	HCO <sub>3</sub> <sup>-</sup>	Cl <sup>-</sup>	Albu min <sup>-</sup>	Globu lin <sup>-</sup>	H <sub>2</sub> PO <sub>4</sub> <sup>-</sup> HPO <sub>4</sub> <sup>-</sup>	Total base	Serum non protein nitrogen	Serum Ca
				m.eq	m.eq	m.eq	m.eq	m.eq	m.eq	mgm per 100 ml	mgm. per 100 ml.
I	-27	VB	0	20.6	104.4	10.0	4.6		153.0		
I	4	VB	0	24.9	108.9	6.4	4.6	2.2	152.0	27	
I	7	VB	0	20.6	111.4	8.2	3.3	3.2	155.8	22	
I	17	VB	++	21.0	114.0	4.3	2.2	3.1	147.0	34	
I	19	AB	++	23.0	112.0	4.7	2.7	2.5	159.5	30	
I	19	VB	++	24.4	111.0	5.0	2.7	2.6	163.0	30	9.9
I	19	AbFl	++	25.2	119.0	0.0	0.0	3.0	158.5	30	7.5
I	23	AB	0	20.8	113.2	8.0	3.0	4.1	151.4	39	
I	23	VB	0	22.5	111.2	8.0	3.0	4.1	152.6	39	
II	-61	AB	0	21.8	111.2	5.9	8.3		151.3	29	
II	-12	VB	0	22.4	112.6	8.8	5.8	3.3	155.8	44	
II	10	AB	++	13.0	133.0	2.8	1.4	4.1	179.1	40	
II	10	VB	++	13.8	133.7	2.9	1.3	4.1	179.4	40	
II	10	AbFl	++	14.2	137.6	0.0	0.0	3.4	165.3	39	
III	1	VB	0	22.7	104.2	11.0	2.5	3.0	156.4	23	12.3
III	2	VB	0	25.7	106.6	12.5	1.6	2.4	159.4	25	12.2
III	3	VB	0	23.9	107.8	9.9	3.0	2.5	166.6	22	11.9
III	8	VB	+	19.6	118.4	7.5	0.4	3.0	165.8	31	9.2
III	11	AB	++	19.6	114.6	4.5	2.3	2.8	145.9	26	8.7
III	11	VB	++	20.6	112.6	4.5	2.8	2.8	151.0	26	9.7
III	11	AbFl	++	22.8	121.4	0.3	0.0	2.2	153.3	26	6.8
IV	1	VB	0	19.2	116.4	11.8	3.4	3.2	164.7		
IV	2	VB	0	10.6	108.4	11.4	1.9	3.2	153.0		13.1
IV	4	VB	0	23.0	115.4	10.4	3.5	2.9	162.0		12.1
IV	5	VB	+-	26.8	110.6	(11.0)		2.7	159.8	39	12.1
IV	6	VB	+-	25.7	113.6	9.2	0.9	2.6	161.6		
IV	7	VB	+-	24.9	112.2	7.6	1.0	2.6	162.8		12.0
IV	8	VB	+-	23.8	114.8	8.7	0.9	2.5	157.6	32	12.1
IV	9	VB	+-	25.1	113.8	7.3	0.8	2.5	157.0	23	
IV	10	VB	+-	24.0	119.4	5.9	0.8	2.7	160.4		
IV	11	VB	+	23.0	115.6	5.1	1.1	2.4	168.2	23	
IV	12	AB	++	20.3	119.2	5.0	(1.5)	3.5	159.0	22	10.4
IV	12	VB	++	22.6	112.2	5.4	1.6		160.8	22	
IV	12	AbFl	++	22.8	126.8	0.0	0.0	2.7	157.4	20	8.4
IV	13	VB	+	24.5	115.2			2.6		31	
IV	14	VB	+-	25.3	111.2	5.7	2.8		160.2		
IV	15	VB	0	28.7	107.4	7.1	3.2	2.2	157.5	26	
IV	18	VB	0	25.9	110.4	6.8	4.0	2.6	157.3	31	12.3
IV	23	VB	0	23.4	109.4	9.3	3.3	1.7	152.4		12.9
IV	32	VB	0	22.9	112.0	10.7	1.8	3.0			11.8

Same dog 11 months later

V	1	VB	0	22.8	105.8	8.8	5.3		151.2	25	
V	7	VB	0	22.9	117.0	6.4	3.6	1.8	162.8		
V	11	VB	+	20.5	118.2	4.3	2.5		159.2		
V	13	AB	+	18.7	121.4	3.5	2.7	2.2	162.8		
V	13	VB	+	19.6	119.6	3.3	2.9	2.2	151.2	22	
V	13	AbFl	+	17.2	125.6	1.9			163.2	26	

\* AB = Arterial blood  
VB = Venous blood  
AbFl = Abdominal fluid

high chloride and total base, and low bicarbonate, the serum in Experiment IV on the second day when the bicarbonate was unexplainably low, and the ascitic fluid in Experiment V which was contaminated with blood and suffered a good deal of exposure before analysis. The results on these samples were not used in the analysis of the data.

The data give one an opportunity to observe the effect of a change in protein concentration on the concentration of bicarbonate, chloride and total base. The general trend of the results can be appreciated best by examining Charts 1 and 2. In Chart 1 the concentrations of bicarbon-

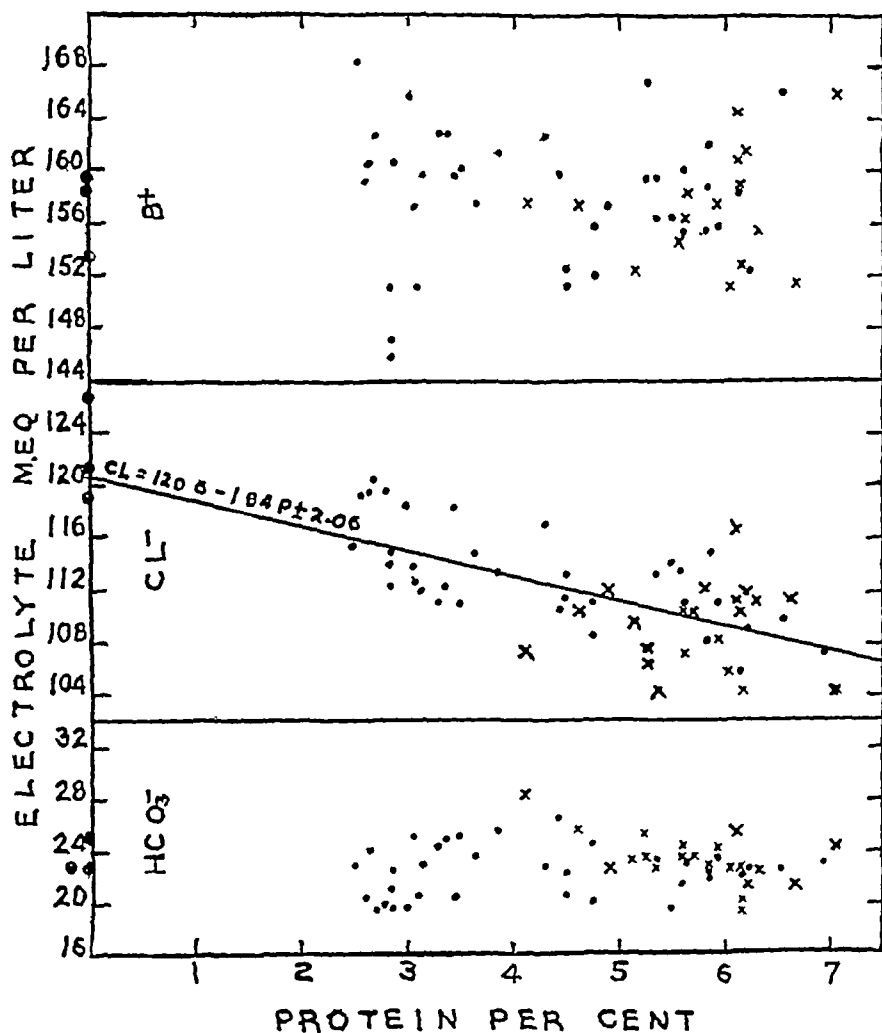


CHART 1

ate, chloride and total base per liter of serum are represented as related to protein per cent. Chart 2 shows the same relationship when all concentrations are expressed per kilogram of serum water. The original values

were converted to concentrations per kilogram of serum water by the formula previously mentioned. The purpose of Chart 2 is to eliminate the effects that are brought about by the varying content of serum water which accompanies the large changes in serum protein concentration. Although the formula for estimating water content from protein per cent expresses dried weight rather than water content, it is probably

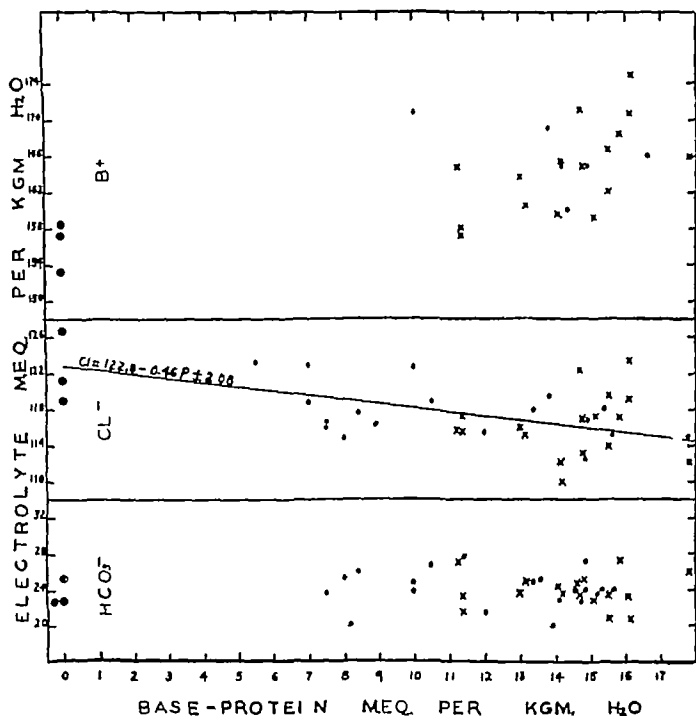


CHART 2

sufficiently accurate for our purpose. In Chart 2, the protein per cent is converted to base combining power, since the base combining power of the proteins is the factor most likely to affect electrolyte concentration if the effect of protein on water content is eliminated. In both charts the data of serums taken over forty hours after any previous administration of salt solution are represented by  $\times$ 's, those of serums taken about eighteen hours after administration of salt solution by dots and those of the



ascitic fluids by dots surrounded by circles. The figures obtained in both the control (see Table II) and the plasmapheresis experiments are represented on the charts. The introduction of the data of the control animals does not significantly alter the interpretation of the results which would be obtained if only the data of the plasmapheresis animals had been used.

Inspection of the charts reveals that the distribution of bicarbonate and total base is not significantly affected by the change in protein concentration. Both bicarbonate and base are somewhat more widely scattered than normal but this dispersion does not seem to be brought about by the injection of salt solution since the  $\times$ 's and dots have about the same distribution.

Chart 1 shows that the chloride varies inversely with the protein. The line of regression<sup>1</sup> is represented on the chart and the equation of this is  $(Cl)_s = 120.64 - 1.87 P \pm 2.06$  where  $(Cl)_s$  is chloride in m eq per liter,  $P$  is protein per cent, and  $\pm 2.06$  is the probable error. The probable error of the slope (or regression coefficient) is  $\pm 0.21$ . The equation was derived without using the data of the ascitic fluids, but it should be noted that the latter are in fairly good agreement with the value predicted by the regression equation for serum with no protein.

Chart 2 shows a similar inverse relationship between chloride and base-protein when the concentrations are expressed per kilogram of serum water. The line of regression is  $(Cl)_s = 122.8 - 0.46 (BP) \pm 2.08$  where  $(Cl)_s$  is chloride in m eq per kilogram of serum water,  $(BP)$  is the base combining power of the serum proteins in m eq per kilogram of serum water and  $\pm 2.08$  is the probable error. The probable error of the slope (or regression coefficient) is  $\pm 0.08$ . The equation predicts a concentration of chloride for a serum with no protein that agrees somewhat better with those of the ascitic fluids than the previous equation.

It will be noticed that the high chloride values occur chiefly in the serums which may be affected by the administration of salt solution. In the plasmapheresis experiments, this necessarily occurred, since it is difficult to maintain a low level of serum protein without continuous bleeding and the accompanying injection of physiological salt solution. However, the chlorides of the serum taken about eighteen hours after the

<sup>1</sup> For the statistical formulas used in the paper, the reader is referred to Dunn (Physiol Rev, 1929, ix, 336). The regression equation is

$$Y - \bar{Y} = r_{xy} \frac{\sigma_y}{\sigma_x} (X - \bar{X})$$

The probable error of the regression coefficient is  $\pm 0.6745 \sqrt{\frac{1 - r_{xy}^2}{n - 2}} \left[ \frac{\sigma_y}{\sigma_x} \right]$

This gives the variations in the slope of the line that may be due to sampling. The probable error of the  $Y$  calculated from  $X$  is  $\pm 0.6745 \sigma_y \sqrt{1 - r_{xy}^2}$ . This gives the variations in the predicted values that may be due to sampling. The probable error of the difference is  $\sqrt{(P.E.)_1^2 + (P.E.)_2^2}$ .

administration of salt solution vary inversely with the proteins. The slope of the line representing this relationship would be somewhat less than the one drawn on the chart. It should be pointed out that if there is increase in chloride brought about only by the effect of the injection of salt solution, there is no evidence of a similar retention of sodium reflected in the values of the total base. Furthermore it can be demonstrated that the increase in chloride is not accompanied by a compensating decrease in bicarbonate although there is evidence of the well recognized inverse relationship between chloride and bicarbonate.

Collateral evidence that there is an indirect relationship between chloride and protein is furnished by the control experiments presented in Table II. These experiments were designed to obtain blood from dogs

TABLE II  
*Electrolyte concentration of serum of control dogs*

Dog number	Day	Weight	HCO <sub>3</sub> <sup>-</sup>	Cl	Albu- min <sup>-</sup>	Globu- lin <sup>-</sup>	H <sub>2</sub> PO <sub>4</sub> <sup>-</sup> HPO <sub>4</sub> <sup>-</sup>	Ca <sup>++</sup>	Total base	Serum pro- tein	Intravenous 0.9 per cent NaCl	
											A.M.	P.M.
		kgm.	m.eq	m.eq	m.eq	m.eq	m.eq	m.eq	m.eq	grams per 100 ml	cc.	cc.
6	1	8.8	24.3	104.4	12.1	4.9		5.9	155.2	7.04	450	
	2	8.8	23.3	107.4	12.2	4.6	4.1	6.2		6.94	350	
	3	8.7	22.5	109.8	10.6	4.9		6.6	156.1	6.55	350	
	4	8.9	22.8	109.0	10.3	4.6		5.8	152.1	6.19	450	
	5	8.6	22.7	108.0	9.9	4.1		5.9	155.8	5.83	475	
	8	8.3	24.3	108.2	8.9	5.0	2.8	5.7	158.0	5.94	475	450
	9	8.3	22.2	105.8			3.0	5.6	158.5	6.12	475	450
	10	8.7	23.5	111.2	8.5	5.3	3.5		155.8	5.92		
	13	8.6	23.8	107.0	8.4	4.7	2.9	5.9	156.5	5.61		
7	1	9.3	22.5	110.4			2.0	5.9	158.3	5.69		
	8	9.4	25.7	110.4			3.0	6.1	158.8	6.11		
	20	9.4	21.9	112.0			2.2	6.1	161.6	6.21		
	31	9.4	22.5	111.6	10.7	3.8	2.6	6.2	161.0	6.10	450	350
	32	9.6	23.8	111.2	9.4	3.3	2.1	5.6	160.7	5.16	400	400
	33	9.0	22.0	112.2	9.4	4.5	2.7	5.6	159.0	5.84	400	400
	34	9.4	21.7	113.4	9.2	4.1	1.8	5.3	155.5	5.56	450	450
	35	9.4	19.4	114.4	9.5	3.8	2.2	5.3	156.5	5.48	450	450
	36	9.6	23.4	113.2	9.7	3.4	1.6	5.3	159.5	5.36		
	41	9.7	24.4	111.0	9.8	3.7	4.0	5.7	154.7	5.60		

subjected to substantially the same procedures as the other animals, excepting plasmapheresis. Dividing the serums from these dogs into one group taken over forty hours after administration of salt solution and another group taken about eighteen hours after administration of salt solution, one may obtain an idea of the magnitude of the error introduced in the plasmapheresis experiments by the injection of sodium chloride

The average values of the two groups indicate that injection of physiological saline was accompanied by a decrease of protein of  $0.13 \pm 0.14$  per cent, a decrease of bicarbonate of  $1.18 \pm 0.38$  m eq per liter and a decrease of total base of  $1.16 \pm 0.74$  m eq per liter, while there was an increase of chloride of  $1.13 \pm 0.81$  m eq per liter. The changes are represented together with the probable errors of the differences. The decrease in bicarbonate is over three times the probable error, but changes in chloride, protein and total base are of about the same magnitude as the probable errors. While the number of determinations is small, the results indicate that it is not likely that a larger number of determinations would show an increase in chloride greater than 2.5 m eq per liter. Since the protein contents of the two groups are so nearly identical, converting the concentrations per liter to concentrations per kilogram of serum water does not significantly alter the results. The equation for chloride per liter indicates that a serum with three per cent protein has 5.6 m eq more chloride than a serum with six per cent protein. Hence although the slope of the line in Chart 1 might be less if some of the values were not affected by injection of salt solution, it is extremely probable that the line represents chiefly the relationship of the chloride to the protein. The equation for the chloride per kilogram of serum water indicates that the chloride of a serum with 7 m eq of base-protein is 3.7 m eq higher than that of one with 15 m eq base-protein. This is a little more than three times the probable difference which the control experiments indicate might be produced by the injections of salt solution. It indicates that further experiments would probably substantiate an inverse relationship between chloride and base-protein per kilogram of serum water. However, the authors prefer to regard this result as only suggestive.

The theoretical significance of the results is not clear. Presumably the chloride increases in serum with low protein in such a manner as to keep the total anion concentration constant. The increase in chloride with decrease in protein can be largely accounted for by the increase of serum water accompanying a decrease in protein. The results suggest that there is a small increase in chloride to compensate for the decrease in the base bound to protein.

It is well recognized that, in subjects with edema accompanied by low serum protein concentration, the serum chloride concentration is usually normal or high. This has been emphasized lately by Peters, Wakeman, Eisenman and Lee (11), Peters, Wakeman and Eisenman (12), Blackfan and Hamilton (13) and others. However, their data show quite irregular variations at any given protein concentration and do not permit one to express the concentration of chloride in terms of concentration of protein. Such a relationship presumably exists in man, but other factors affect the chloride in cases of nephritis and nephrosis so that the relationship is not clear cut in data from such patients. In cases of nephrosis and

hydropigenous nephritis, the concentration of total base and, to a lesser degree, that of the bicarbonate are low. Such a change was not demonstrated in the plasmapheresis experiments.

## *II A comparison of the concentrations of electrolytes of ascitic fluid with those of arterial and venous serum*

There is little ground for assuming that a particular sample of blood is in equilibrium with ascitic fluid. To assume such an equilibrium, even approximately, the blood sample should represent the blood in the capillaries supplying the peritoneum. However, if peritoneal fluid is present in large quantities, its composition must represent an equilibrium with the average venous plasma returning from this area during the preceding few hours. An average arterial sample of plasma probably represents quite accurately the plasma supplying the peritoneum, but the venous samples that can be obtained differ from the venous plasma which is theoretically required by an amount which cannot be determined. Nevertheless, since it is desirable to know how truly plasma represents the immediate environment of cells, it is interesting to see what relationship holds between ascitic fluid and arterial and venous plasma.

The data on the ascitic fluids and arterial and venous plasma in the first four experiments are fairly suitable for this purpose. In Experiment II, the failure to get blood into the vein after withdrawal of a rather large quantity of blood was followed by symptoms of shock and acidosis. The samples were obtained only six hours after the administration of salt solution which accompanied the transfusion of cells and probably the ascitic fluid had not had time enough to come into complete equilibrium. Furthermore, the high concentration of chloride indicates that the dog had not been able to bring the composition of his blood back to normal. Though the dog appeared to be recovering, he was killed because he seemed so uncomfortable. The data of this experiment are included because they are similar to the rest in most respects. Contamination with blood and exposure to air rendered the ascitic fluid in Experiment V unsuitable.

Table III gives the distribution ratios for total base, bicarbonate, chloride, and bicarbonate plus chloride. All concentrations were expressed in m eq per kilogram of water in calculating the ratios. Since protein is largely indiffusible through capillary membranes, the concentration of protein in the ascitic fluid and blood plasma is the chief factor which should alter the distribution ratios of the freely diffusible ions. However, it is known that the distribution of calcium is altered by the fact that calcium appears to be bound to protein in an undissociated compound. Moreover, Loeb, Atchley and Palmer (14), Hastings et al (15) and Greene et al (16) found that the concentration of serum potassium is considerably higher than that of ascitic fluid. One does not know

the effect of protein on the activity of the various ions, though Greene et al have calculated the ionized base by assuming that the chloride ratios are true Donnan ratios. Until these factors are known, a theoretical ratio based on the concentration of protein and the assumption that the

TABLE III  
*Distribution ratios of serum and ascitic fluid (m eq per kilo H<sub>2</sub>O)*

Experiment number	Sample *	$r$ *	$\frac{(B^+)_f - 2(Ca^{++})_f}{(B^+)_s - 2(Ca^{++})_s}$	$\frac{(HCO_3^-)_s}{(HCO_3^-)_f}$	$\frac{(Cl^-)_s}{(Cl^-)_f}$	$\frac{(HCO_3^-)_s + (Cl^-)_s}{(HCO_3^-)_f + (Cl^-)_f}$
I	AB	0.979	0.97	0.94	0.98	0.97
I	VB	0.975	0.94	1.00	0.97	0.97
II	AB	0.988	0.91	0.94	0.99	0.98
II	VB	0.988	0.91	0.99	0.99	0.99
III	AB	0.978	1.02	0.89	0.98	0.96
III	VB	0.976	0.99	0.93	0.96	0.96
IV	AB	0.979	0.97	0.92	0.97	0.95
IV	VB	0.977	0.95	1.02	0.92	0.93

\* AB = Arterial blood

VB = Venous blood

$$r = \frac{(B^+)_f - 2(Ca^{++})_f}{(B^+)_f - 2(Ca^{++})_f + 1/2(BP)_s}$$

(BP)<sub>s</sub> = Base combining equivalent of serum protein

conditions for a true Donnan equilibrium exist can have only an empirical value. However, the desirability of expressing the relationship existing between plasma and interstitial fluid in terms of serum protein and total base justifies the use of such an empirical ratio until a better expression for this relationship is found.

In the tables, the ratio,  $r$ , was calculated for comparison with the ratios found. It is based on the assumptions made by Van Slyke (17) in calculating similar ratios and the formula used is as follows:

$$r = \frac{(B)_f - (Ca)_f}{(B)_f - (Ca)_f + 0.5 (BP)_s}$$

in which (B)<sub>f</sub> is the total base of ascitic fluid, (Ca)<sub>f</sub> is calcium of ascitic fluid and (BP)<sub>s</sub> the base combining power of the serum proteins, all concentrations being expressed in m eq per kilogram of water. The calcium is subtracted to reduce the base to univalent terms. Ascitic base was thought to represent average conditions better than venous serum base, since a large amount of ascitic fluid is probably less subject to sudden temporary deviations from average conditions. However, as pointed out previously, the ratio,  $r$ , does not take into account the peculiar distribution of calcium and potassium, the presence of a probably insignificant amount of magnesium and probably other factors.

The actual ratios agree approximately with each other and with the ratio,  $r$ . The agreement is best with respect to chloride and chloride plus bicarbonate, but is only fair with respect to bicarbonate and base. It is of interest that, in Experiment II, where the blood was taken 6 hours after injection of salt solution and apparently before the blood plasma had had time to come into equilibrium with the ascitic fluid, the chloride and bicarbonate ratios show about the same relation to  $r$  as in the other experiments, while the base is still apparently too high in the plasma. This would indicate that the concentrations of bicarbonate and chloride can be adjusted more rapidly than base.

The experiments give about as good agreement in the ratios as those in the literature (14, 15, 16, 18, 19). However, the ratios can not be used to predict the composition of ascitic, and presumably interstitial fluid in general, except as a first approximation. The wide deviation of the venous base ratio in Experiment I, of the arterial and venous bicarbonate ratios in Experiment III, and of the venous chloride ratios in Experiment IV indicate that difficulty in obtaining average blood samples is one of the sources of error. It should also be pointed out that all the ratios are quite near unity and, therefore, have considerably less value than they would have if lower. However, means have not been found to obtain interstitial fluid differing greatly in protein concentration from plasma.

### *III The effect of serum protein concentration on serum calcium concentration*

Chart 3 shows the relationship between serum protein and serum calcium. Assuming that ascitic fluid has the composition that plasma would have if free of protein, the determinations on ascitic fluid are included with those on serum. Chart 3 shows that calcium varies directly with the protein. The correlation coefficient of the calcium and protein is  $0.93 \pm 0.03$ . The regression equation is as follows:  $(Ca) = 0.94 P + 7.45 \pm 0.44$ , in which  $(Ca)$  is the calcium concentration in mgm. per 100 ml,  $P$  is the protein per cent and  $\pm 0.44$  is the probable error. The probable error of the regression coefficient is  $\pm 0.06$ . When the protein concentration is zero, this would indicate a calcium concentration of 7.45 mgm per 100 ml which agrees well with the average value (7.5) of the ascitic fluids.

The direct relationship of calcium and protein concentration has been pointed out by Salvesen and Linder (20), Marrack and Thacker (21) and Peters and Eiserson (22). It is realized that calcium concentration is affected by the concentration of phosphate, the activity of the parathyroid glands, the amount of activated ergosterol in the body and probably other factors. The variations in phosphate in our experiments are too small to account for the differences in calcium. The data are so clear cut that they leave little doubt as to the direct relationship between calcium and protein concentration.

Recently Stearns and Knowlton (23) analyzed the relationship between serum protein, calcium and phosphate in 76 infants and children. They concluded that there was no relationship between serum protein and calcium in non-nephritic cases. However, their data are made up of (1) a group of cord bloods, (2) a group of bloods from infants fed cod liver oil and who were under a year old, and (3) a group of children over a year old. For analysis these groups are of different value. The data show no significant correlation between the calcium and protein in the newborn cord blood or that from the children over one year old. Cord blood is not altogether satisfactory for chemical studies and probably

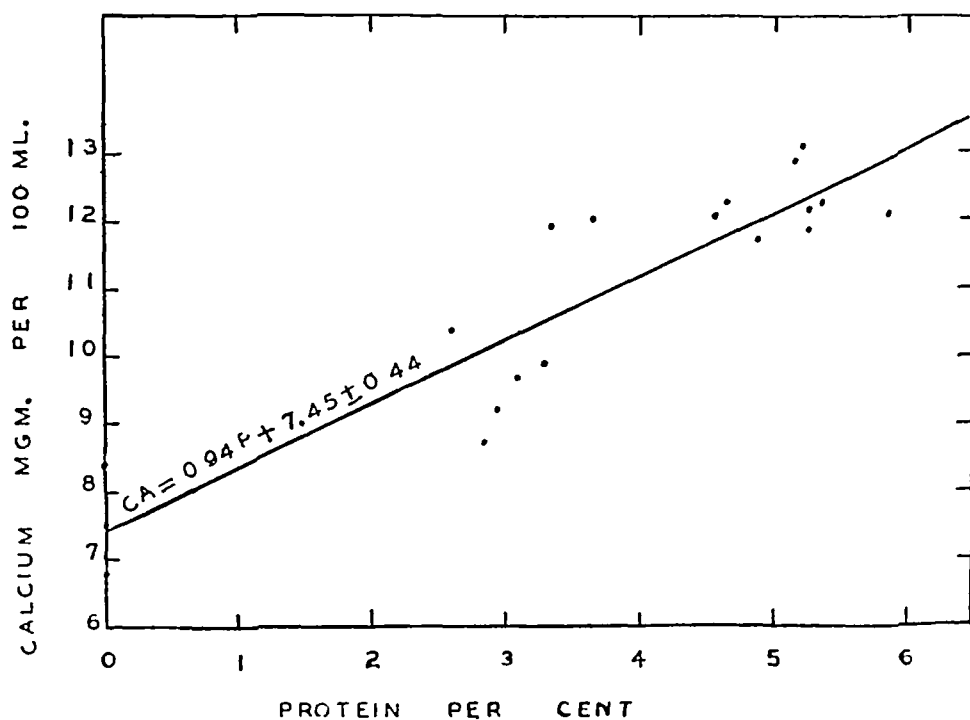


CHART 3

should be used with caution. In the group of forty-five children's serums, only one showed a very low protein concentration and this one had a normal calcium concentration. All that can be concluded from this group of cases is that variations in the calcium do not correlate with variations in the protein when there is only a small change in the protein concentration. However, the serums of the babies are quite satisfactory since they represent a uniform group from sixteen patients who had received cod liver oil and manifested a fairly wide distribution of protein. The correlation coefficient of the serum calcium and protein is  $0.64 \pm 0.11$ . The regression equation for this group is  $Ca = 0.41P + 8.87 \pm 0.25$ . The probable error of the slope (or regression coefficient) is  $\pm 0.22$ . The

equation of Peters and Eiserson (22) obtained from a mixed group with many cases of nephritis is  $Ca = 0.556 P + 6$ . Since the regression coefficient of the equation for the babies is  $0.41 \pm 0.22$ , the slope of this line cannot be considered to differ significantly from that of the line found by Peters and Eiserson. However, the constant in the latter equation differs significantly from that for the babies. The higher value for the constant in the infants presumably represents chiefly the effect of activated ergosterol in growing infants. Further data will probably show that the equation of Peters and Eiserson can be used to measure the effect of factors other than protein which modify the calcium concentration. Peters and Eiserson's equation for phosphate probably does not apply to patients fed large amounts of activated ergosterol. The general form of the equation would be  $Ca = 0.556 P + K$ . It will probably be helpful to see how far the  $K$  necessary to fit any given data can be used as a measure of the effect of the factors besides protein concentration which affect the level of serum calcium.

#### SUMMARY

1 Electrolyte studies are reported on the serum and ascitic fluids of dogs rendered edematous by lowering the plasma protein concentration by plasmapheresis.

2 The concentrations of total base and bicarbonate show no relation to protein concentrations under the conditions of the experiments. The total base values are quite widely scattered (145 to 168 m eq per liter or 150 to 175 m eq per kilogram of serum water). The bicarbonate concentrations are also quite variable (19 to 28 m eq per liter or 20 to 29 m eq per kilogram of serum water). The control experiments demonstrated that the injection of salt solution reduced the bicarbonate and rendered these values in the plasmapheresis experiments unreliable as a reflection of change in protein concentration. While it was not demonstrated that the injection of salt solution was likely to alter the base concentration, the wide scattering of the base concentrations was probably brought about by a combination of factors inherent in the plasmapheresis experiments.

3 The concentration of chloride per liter varies inversely with that of protein according to the following regression equation  $(Cl)_s = 120.64 - 1.87 P \pm 2.06$  in which  $(Cl)_s$  is the chloride in m eq per liter,  $P$  the protein per cent and  $\pm 2.08$ , the probable error.

4 The data show that chloride m eq per kilogram of serum water varies inversely with that of base protein according to the following regression equation  $(Cl)_s = 122.8 - 0.46 (BP)_s \pm 2.08$  in which  $(Cl)_s$  is chloride m eq per kilogram of serum water,  $(BP)_s$  is the base combining power of serum protein in m eq per kilogram of serum water and  $\pm 2.08$  the probable error.



5 The control experiments indicate that the experimental procedures were not the chief cause of the appearance of the relation expressed in the chloride equation which gives concentrations per liter of serum except inasmuch as they varied the protein concentration. However, the change in chloride per kilogram of serum water lies within the increase in chloride which might be produced by injecting salt solution. The latter equation, though likely to be substantiated, cannot be accepted as expressing a relationship between chloride and protein at present. The slope of the line in the first equation would probably be a little less if derived from experiments not affected by the injection of salt solution but the change is not likely to alter the equation greatly.

6 The ratios

$$\frac{(Cl^-)_s}{(Cl^-)_f}, \frac{(HCO_3^-)_s}{(HCO_3^-)_f}, \frac{(B^+)_f - (Ca)_f}{(B^+)_s - (Ca)_s}$$

agree approximately with each other. However, there is sufficient diversity in the ratios to demonstrate that a given specimen of serum is not likely to predict the composition of ascitic fluid very accurately.

7 The calcium and protein concentrations vary directly with each other according to the following regression equation  $(Ca) = 0.94 P + 7.45 \pm 0.44$  in which  $(Ca)$  is serum calcium in mgm per 100 ml,  $P$  the protein per cent and  $\pm 0.44$  the probable error.

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# THE PASSAGE OF FLUID AND PROTEIN THROUGH THE HUMAN CAPILLARY WALL DURING VENOUS CONGESTION

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It has been demonstrated in normal human subjects by several methods that during venous congestion fluid is filtered from the blood into the tissue spaces. This passage of fluid through the capillary wall has been identified by comparing blood samples removed during normal circulation and during venous congestion (Schultz and Wagner (1909), Rowe (1915), Dautrebande, Davies and Meakins (1923), Peters, Eisenman and Bulger (1925)). It has been observed also that when the venous pressure in the legs is increased by quiet standing, fluid is filtered from the blood stream with a consequent reduction in circulating blood volume (Thompson, Thompson and Dailey (1928), Waterfield (1931)). The filtration produced by measured grades of venous congestion has been measured also by plethysmographic methods in which the accumulation of fluid in the tissue spaces was estimated by measuring the increase in limb volume (Drury and Jones (1927), Krogh, Landis and Turner (1932)).

The capillary wall has usually been regarded as relatively impermeable to proteins (Krogh (1929), Thompson, Thompson and Dailey (1928), Krogh, Landis and Turner (1932)). Waterfield (1931) however, differed from Thompson, Thompson and Dailey in finding that during standing the blood lost not only fluid but also a significant amount of protein. Drinker and his co-workers (1931) have called attention to the high concentration of protein often found in lymph. Maintaining that tissue fluid and lymph are identical, they regard the capillary wall as everywhere quite permeable to protein even under normal conditions.

A retrograde movement of protein from the tissue spaces through the capillary wall into the blood during venous stasis has been described by Plass and Rourke (1927). They found that during venous congestion the blood proteins were always increased by a greater percentage than was cell volume. This was believed to indicate that during venous congestion proteins must pass from the tissue spaces into the blood stream. It will be shown below that the direct comparison of percentage increase in cell volume with the percentage increase in plasma proteins is unjusti-

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fied. Actually their figures show loss of both fluid and protein from the blood stream.

The amount of tissue fluid accumulating in the arm during mild venous congestion (Krogh, Landis and Turner (1932)) seemed great enough to produce measurable changes in the fluid content of the blood. Assuming that the fluid is lost chiefly from the plasma, the amount of capillary filtrate per 100 cc. of blood can be calculated from the cell volume of normal blood and the apparent increase in the cell volume of the blood after passage through the congested vessels.

If the capillary wall were totally impermeable to colloids the protein content of the blood would be increased in proportion to the reduction in plasma volume. However, in computations involving changes in cell volume and plasma proteins, allowance must be made for the fact that the cell volume is measured in volumes per cent of blood (i.e. plasma plus cells) while blood proteins are measured in terms of percentage of plasma. Knowing the change in cell volume produced by stasis and the protein percentage of normal blood, it is possible to calculate the increase in plasma protein to be expected if no protein were lost in the capillary filtrate. The difference between the calculated amount of protein and the amount determined by analysis indicates the amount of protein lost in the capillary filtrate.

From a recalculation of the data reported by Plass and Rourke (1927), and from our own findings, it appears that high grades of venous congestion caused the filtration of relatively large amounts of fluid containing a high percentage of the three protein fractions present in blood plasma. Very low congestion pressures were accompanied by the filtration of small amounts of fluid without detectable loss of protein, but the volume changes were probably too small to permit any certain conclusion concerning protein. Finally, in two patients edema fluid largely produced by, and collected during, venous stasis contained 0.39 and 0.09 per cent protein.

#### METHODS

The blood studies were made in normal male subjects or in male patients hospitalized for minor maladies unrelated to the circulatory system. Their blood pressures were not in any instance significantly abnormal, their ages ranged from 24 to 40 years.

In order to avoid postural changes in fluid balance the subjects reclined 30 minutes before the venous congestion was started. The forearms were supported on sandbags at the side of the body, so that the upper (flexor) surfaces were level with the clavicles. The skin temperature of each forearm was measured at five-minute intervals by means of thermal junctions held, by a single layer of surgeon's plaster, in contact with the skin on the flexor surface of the forearm midway between wrist and elbow. Venous pressure was elevated by means of Riva Rocci armlets, 12 cm. wide and 50 cm. long, wrapped around the arm well above the elbow.

At the end of the thirty minute rest period the armlets were inflated simultaneously and suddenly from reservoirs connected with manometers. On the

control side the armlet pressure was 9 mm Hg, a pressure which, though enough to distend the veins slightly, did not produce measurable filtration in plethysmographic observations (Krogh, Landis and Turner (1932)) The pressure in the other armlet was raised to 20, 40, 60, or 80 mm Hg in the experimental observations and to 9 mm Hg in three control observations. The subjects were cautioned against moving the arm or forearm during the congestion period.

When venous pressure had been elevated for a period of 30 minutes samples of venous blood were removed simultaneously from each arm, approximately 30 cc. of blood being collected in a syringe containing 20 mgm of dry heparin. The armlets were deflated only after the blood samples were removed. The blood was agitated in the syringes for fifteen minutes to dissolve the heparin and to mix it thoroughly with the blood. The samples were then transferred to test tubes in which the gentle agitation was continued while small amounts were removed for determinations of cell volume, hemoglobin and erythrocyte counts.

Hematocrit determinations were carried out immediately, usually in triplicate, using tubes having an inside diameter of 2 mm and a length of 12 cm. A certain number of determinations were also made with Wintrobe hematocrit tubes. The filled tubes were rotated for 30 minutes at a speed of 3000 r p m.

#### PROTOCOL 1

*Venous congestion of 80 mm Hg*

*Subject L December 8, 1931 Room temperature 24.2° C*

Time	Arm temperature		Air temperature	Notes
	Right	Left		
p m.	C.	C.	C.	
6:40				Subject reclined thermal junctions applied and armlets placed in position
7:10	33.7	33.4	24.1	Venous congestion begun
7:15	33.9	33.4	24.3	80 mm. Hg on left arm
7:20	33.9	33.1	24.2	9 mm Hg on right arm
7:25	33.9	32.7	24.2	
7:30	33.8	32.6	24.2	
7:35	33.7	32.3	24.3	Thermal junctions removed
7:40				Blood sample of 30 cc. removed from each arm into syringe containing 20 mgm of heparin

#### Blood studies

Congestion pressure	Erythrocytes	Cell volume	Total protein	Globulin	Albumin	Nonprotein nitrogen
mm Hg	per c. mm	per cent	grams per 100 cc.	grams per 100 cc.	grams per 100 cc.	grams per 100 cc.
9	5,596,000*	53.1†	6.58	2.43	4.15	0.35
Observed 80	6,334,000*	60.2†	7.81	3.02	4.79	0.34
Calculated 80			8.71	3.32	5.49	

\* Average of 8 counts

† Average of 3 tubes

In certain observations 3 to 5 erythrocyte counts were made on each sample by each of two observers, the results being averaged to determine the percentage increase in erythrocytes produced by stasis. Hemoglobin determinations were made according to the method of Dreyer, Bazett and Pearce (1920). Of the blood 0.1 cc. was diluted with 19.9 cc. of 0.9 per cent sodium chloride solution. This suspension of cells was hemolyzed with saponin and the depth of color in the two specimens was compared by colorimeter, using diffuse daylight. The blood from the control side was taken as 100 per cent and only the relative increase was determined. Plasma proteins were estimated according to the method of Howe (1921). When the changes in total protein were conspicuously large, the plasma proteins were separated into globulin and albumin fractions, the former containing also fibrinogen.

Protocols 1 and 2 show the details of experiments at congestion pressures of 80 mm. Hg and 40 mm. Hg respectively.

#### PROTOCOL 2

*Venous congestion of 40 mm. Hg*

*Subject R December 29, 1931 Room temperature 24.5° C*

Time	Arm temperature		Air temperature	Notes
	Right	Left		
<i>p m</i>	<i>° C</i>	<i>° C</i>	<i>° C</i>	
6.39				Subject reclined, thermal junctions applied and armlets placed in position
7.09	34.4	33.7	24.8	Venous congestion begun
7.14	34.4	33.0	24.8	40 mm. Hg on left arm
7.19	34.3	33.0	24.2	9 mm. Hg on right arm
7.24	34.3	32.9	24.3	
7.29	34.2	32.9	24.3	
7.34	34.1	32.7	24.3	Thermal junctions removed
7.39				Blood sample of 30 cc. removed from each arm into syringe containing 20 mgm. of heparin

#### Blood studies

Congestion pressure	Hemoglobin	Cell volume	Total protein	Nonprotein nitrogen
<i>mm. Hg</i>	<i>per cent initial</i>	<i>per cent</i>	<i>grams per 100 cc.</i>	<i>grams per 100 cc.</i>
9	100.0	38.8*	6.17	0.28
Observed, 40	106.3	41.0*	6.80	0.26
Calculated, 40			6.79	

\* Average of two tubes

#### METHODS OF CALCULATION

To calculate the loss of fluid per 100 cc. of blood from hematocrit determinations requires the assumption that the fluid is lost entirely from the plasma, and that in the concentration of the blood the absolute size of the cellular elements has not changed significantly. Dautrebande, Davies and Meakins (1923) describe a single observation in which stasis increased the hematocrit reading by a smaller percentage than the oxygen capacity and the hemoglobin

content They calculated that 20 per cent of the fluid lost by the blood must have been removed from the erythrocytes and 80 per cent from the plasma Peters, Eisenman and Bulger (1925), on the contrary, report two observations in which the application of a tourniquet to the arm for five minutes increased the oxygen capacity by 20.6 and 23.3 per cent while the cell volume was increased by 19.9 and 25.5 per cent respectively They conclude that the fluid lost from the blood during stasis is removed almost entirely from the plasma A similar finding is reported by Peters (1924)

Van Slyke, Wu and McLean (1923) observed that as blood changes from the arterial to the venous state there is a slight but definite increase in cell volume In venous congestion the increase in  $\text{CO}_2$  tension of the blood tends to increase the cell volume which must, of course, affect the hematocrit readings The effect is to some extent counteracted by the increased oxygen saturation This change, though present, is small, amounting to only 0.6 volumes per cent for a change of 30 mm in  $\text{CO}_2$  tension even when oxygen saturation is unchanged (Eisenman, Bulger and Peters (1926)) It did not seem necessary nor advisable to collect the blood under oil since the loss of  $\text{CO}_2$  would only diminish any change of cell volume that had resulted from the accumulation of  $\text{CO}_2$  which occurred during stasis

During venous congestion the concentration of the plasma protein increases If the wall of the erythrocytes were impermeable to protein alone this change might be expected to diminish cell volume. Van Slyke, Wu and McLean, however, on the assumption that the membrane of the erythrocyte is impermeable to proteins and to the inorganic cations, regarded the osmotic effects of the plasma proteins as a negligible factor in the movement of water between the erythrocytes and plasma because the effective osmotic pressure of the plasma can be modified very little by change in the plasma protein percentage

Sudden variations in the number of red cells per unit volume of blood have been described by Lamson, Abt, Oosthuisen and Rosenthal (1923) Since the arterial blood entering both arms must have the same composition, systemic variations in red cell number would be present in both arms equally To avoid the effects of these changes blood samples were removed from the control and the experimental arms simultaneously and at approximately the same rate In this way each experiment included its own control The possible effects of systemic variations in red cell number on the comparison of unconcentrated and concentrated blood were thereby avoided

In all the observations here reported the hematocrit readings were matched with either red cell counts or with hemoglobin determinations to identify any significant error which might arise from changes in absolute cell volume No consistent differences were found (Table 1) between the two series of figures Under the conditions of these observations, therefore, it seemed justifiable to calculate water loss from the changes in hematocrit readings, assuming that there was no change in the water content of the erythrocytes Thus, if 100 cc. of unconcentrated blood from the control arm is compared with concentrated blood removed simultaneously from the other arm certain relations will hold If the percentile cell volume of the normal blood is  $C_1$  and that of the concentrated blood  $C_2$  while the loss of water or volume from 100 cc. of initially unconcentrated blood is  $x$ , then

$$x = 100 - 100 \frac{C_1}{C_2}$$

If the capillary wall has allowed no plasma protein to pass during the process of concentration of the blood, the absolute amount of plasma protein will be



the same, but the percentage will be increased in proportion to the concentration of the plasma fraction of the blood. If the protein percentage of the normal plasma is  $Pr_1$  and that of the concentrated plasma  $Pr_2$ , while  $Pl_1$  and  $Pl_2$  represent the plasma volume of normal and concentrated blood respectively per 100 cc of initially unconcentrated blood, then, if no gain or loss of water by the cells is postulated,  $Pl_2 = Pl_1 - \nu$ , and

$$Pr_2 = Pr_1 \frac{Pl_1}{Pl_1 - \nu}$$

By comparing the calculated protein percentage,  $Pr_2$ , with that actually observed,  $Pr_2'$ , the loss of protein may be detected. The actual amount of protein lost by the plasma from an initial volume of 100 cc of blood,  $\Delta \overline{Pr}$ , can be estimated from the observed protein percentages

$$\Delta \overline{Pr} = Pr_1 \times \frac{Pl_1}{100} - Pr_2' \times \frac{Pl_1 - \nu}{100}$$

Knowing the amount of fluid ( $\nu$ ) which left the plasma and the simultaneous loss of protein ( $\Delta \overline{Pr}$ ) the percentage of protein in the capillary filtrate can be estimated

#### OBSERVATIONS

The results are collected in Table 1. Room temperature was kept between 22.6 and 25.9° C to avoid marked cooling of the exposed arms during the observation. The average arm temperature varied between 35.1 and 31.6°. Skin temperature fell gradually when higher grades of venous congestion were used, this fall was never more than 1.2° C and was usually less than 1.0° C.

##### (A) *The loss of fluid from the blood during venous congestion*

With a venous pressure of 80 mm Hg the relative cell volume was increased by 13.4 to 24.2 per cent while the hemoglobin and red cells were increased by 13.1 to 22.9 per cent. These changes indicate a loss of fluid amounting to between 11.9 and 19.5 cc from 100 cc of whole blood.

With a venous pressure of 60 mm Hg relative cell volume was increased by 7.8 to 9.7 per cent while red cells and hemoglobin were increased by 7.3 to 10.8 per cent, indicating that the fluid lost amounted to between 7.2 and 8.9 cc from 100 cc of whole blood.

With a venous pressure of 40 mm Hg the changes were still smaller and, due probably to the relatively greater error, more variable. Relative cell volume increased by 1.9 to 5.7 per cent while the hemoglobin increased by 0.9 to 6.3 per cent, indicating that the fluid loss amounted to between 1.9 and 5.6 cc from 100 cc of whole blood.

With a venous pressure of 20 mm Hg there was a measurable change in two of three experiments. Relative cell volume increased by 1.0 to 2.6 per cent and the hemoglobin by 0.7 and 1.6 per cent. The loss of fluid amounted to between 0 and 2.3 cc from 100 cc of whole blood.

In three observations in which venous pressure was 9 mm Hg in both arms there was no change in the relative cell volume in two experiments, and a small but unimportant change in the third

Plass and Rourke (1927) have reported a series of observations in which hematocrit readings were made on blood drawn with and without venous congestion. Those experiments in which measured grades of venous congestion were used have been collected in Table 2 to show fluid loss for comparison with our own data. Venous congestion of 100 mm Hg for 15 to 22 minutes was accompanied by a loss of between 18.9 and 29.3 cc per 100 cc of blood. At 90 mm. Hg the loss was distinctly less, amounting to between 12.5 and 15.4 cc. per 100 cc. of blood. One observation at a venous pressure of 80 mm Hg showed a loss of 7.9 cc. after 26 minutes. The two series of observations indicate that the loss of fluid increases as the venous pressure rises.

The relationship between fluid loss and venous pressure is shown in Figure 1. It is apparent that the loss of fluid is not directly propor-

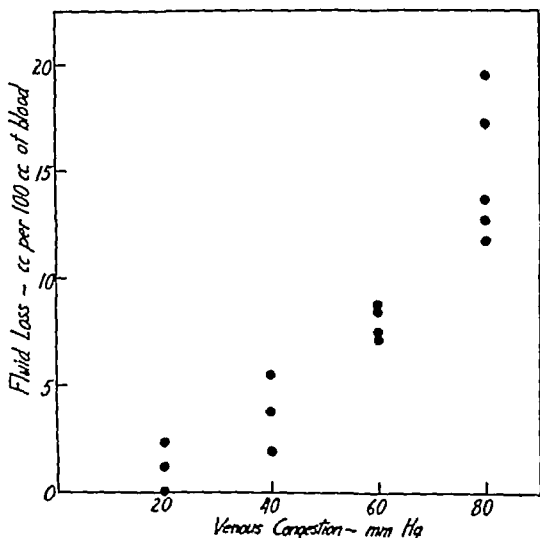


FIG 1 CHART SHOWING RELATIONSHIP BETWEEN VENOUS CONGESTION AND LOSS OF FLUID FROM THE BLOOD

tional to the level of venous pressure. The reason for this is discussed below.

TABLE 1  
Experimental data

Observation number	Room temperature °C	Congestion pressure mm Hg	Arm temperature °C	Cell volume per cent	Erythrocytes millions per c mm	Hemoglobin per cent initial	Increase in		Hemoglobin per centile	Total protein (observed) grams per 100 cc	Total protein (calculated) grams per 100 cc	Calculated minus observed protein grams per 100 cc	Fluid loss cc per 100 cc	$\Delta$ Protein loss grams per 100 cc	Protein in filtrate grams per 100 cc
							Cell volume	Erythrocytes							
2	21.5	9 80	32.4 32.4	39.1† 47.2†				percentile		5.61 7.35	7.82	0.17	17.2	20	1.2
3	23.8	9 80	31.9 32.6	42.7 49.5	4.579 5.243					6.49 8.15	8.53	0.38	13.7	17	1.2
4	21.2	9 80	33.7 32.9	51.3 58.2	5.596 6.334					6.58 7.81	8.71	0.90	11.9	33	2.8
5	23.5	9 80	32.6 32.7	44.5 51.1	4.541 5.343					6.21 8.06	8.09	0.03	12.9	0.1	0.1
16	21.7	9 80	32.6 31.4	42.9 53.3		100.0 122.9				6.81 9.16	10.34	1.18	19.5	44	2.3
6	21.9	9 60	33.6 33.0	46.1 50.6	5.256 5.639					7.06 8.32	8.46	0.14	8.9	0.6	0.7
7	25.2	9 60	34.0 34.0	38.6 41.7	3.987 4.418					6.33 7.24	7.20	-0.04*	7.4	0.0	0.0
8	25.9	9 60	33.4 33.4	46.2 49.8		100.0 108.4				6.69 7.68	7.72	0.04	7.2	0.2	0.3
17	21.8	9 60	32.6 31.5	44.1 48.2		100.0 110.2				6.26 7.36	7.38	0.02	8.5	0.1	0.1

TABLE 1—Continued

Observation number	Room temperature	Congestion pressure	Arm temperature	Cell volume	Erythrocytes	Hemo-globin	Increase in		Hemo-globin	Total protein (observed)	Pr <sub>2</sub> Total protein (calculated)	Calculated minus observed protein	Fluid loss	ΔF Protein loss	Protein in filtrate
							Cell volume	Erythrocytes							
9	24.5	9 40	34.3 32.9	38.8 41.1	millions per c mm.	per cent initial	per cent	per centile	6.3	6.17 6.80	6.79	-0.01*	5.6	00	0.0
10	24.0	9 40	34.3 31.1	48.5 50.4		100.0 104.5	3.9		4.5	6.64 7.49	7.17	-0.32*	3.8	00	0.0
12	25.5	9 40	35.2 33.2	40.6 41.4		100.0 100.9	1.9		0.9	6.55 7.02	6.77	-0.25*	1.9	00	0.0
18	24.0	9 20	32.2 31.6	48.7 49.2		100.0 100.7	1.0		0.7				1.1		
19	24.2	9 20	34.6 33.5	46.3 47.4		100.0 101.6	2.6		1.6				2.3		
20	24.0	9 20	31.5 32.2	51.1 51.1			0.0						0.0		
13	22.6	9 9	34.1 34.1	49.7 49.7		100.0 100.5	0.0		0.5	6.43 6.38	6.43	0.05	0.0		
14	25.0	9 9	31.6 32.3	47.2 47.2		100.0 99.8	0.0		-0.2	6.81 6.75	6.81	0.06	0.0		
15	24.8	9 9	33.3 33.5	44.4 44.2		100.0 99.5	-0.4		-0.5	7.11 7.13	7.06	-0.07	-0.4*		

\* Apparent gain instead of loss

† Equal amounts of sodium citrate used as anti coagulant.

(B) *The passage of protein through the capillary wall during venous congestion*

The observations on the influence of venous congestion on the plasma protein percentage are shown in Table 1. The total protein content of the blood removed from the control arms (venous pressure 9 mm Hg) varied between 5.61 and 7.13 per cent. In the same column below each normal figure is shown the protein content of the blood removed simultaneously from the other arm in which venous pressure was elevated to 80, 60, 40, 20, or 9 mm Hg. For purposes of comparison in the next column are given figures which represent the concentration of protein which, if the capillary walls were impermeable to protein, should have been produced by the fluid loss observed.

In one of the five experiments at 80 mm Hg pressure the observed and calculated protein concentrations agreed, indicating that little or no protein had been lost. In the remaining four experiments the calculated protein content was greater than the observed protein content by 0.38 to 1.18 per cent indicating a significant loss through the capillary wall.

Knowing the amount of plasma, the apparent loss of protein from 100 cc of blood was computed according to the method described above. Dividing the protein loss (grams) by the amount of filtrate formed simultaneously provided a rough estimate of the protein percentage of the capillary filtrate. With a venous pressure of 80 mm Hg the filtrate appeared to contain between 0.1 and 2.8 per cent protein, indicating that under those conditions the capillary wall is far from impermeable to protein.

A venous pressure of 60 mm Hg produced a significant loss of protein in one experiment (number 6), the discrepancy between the observed and calculated protein percentage amounted to 0.14 per cent, corresponding to 0.7 per cent protein in the 8.9 cc of filtrate removed from 100 cc of blood. In the remaining three experiments at this pressure no really significant discrepancy existed between the observed and calculated values. Had 8.0 cc of capillary filtrate containing even 1 per cent of protein been filtered from blood with a plasma volume of 50 per cent, the discrepancy between observed and calculated protein should have been 0.16 per cent. This difference, if actually present, should have been detected, since the control analyses of protein (Experiments 13, 14, and 15) agreed within 0.02 to 0.06 per cent. The capillary filtrate produced at a venous pressure of 60 mm Hg contains, therefore, relatively little protein, averaging in four experiments 0.3 per cent.

The amount of fluid filtered from the blood by a venous pressure of 40 mm Hg is small in comparison with the combined errors of the hematocrit and protein determinations. While no loss of protein was to be detected, this finding is of no significance since, had the filtrate con-

tained 3 per cent protein, the changes in two experiments, (numbers 10 and 12) at least, would have been barely outside the error of the method of protein determination. The apparent gain of protein observed at 40 mm Hg is probably to be explained on the basis of the relatively large errors involved. On this account, protein analyses were not carried out in the three observations in which venous pressure was only 20 mm Hg.

The loss of protein at 80 mm Hg is contrary to the interpretation of Plass and Rourke who concluded from similar experiments at 80, 90 and 100 mm Hg that protein must pass into the blood from the asphyxiated tissue cells. This conclusion was based on their finding that the percentage increase in plasma protein was uniformly greater than the percentage decrease of plasma volume. This direct comparison of percentage changes is, however, not justified since changes in cell volume and plasma volume are computed as volumes per cent of whole blood, whereas protein content is expressed in terms of grams per 100 cc of plasma. The situation can be made clear by considering the change in relative plasma volume and the change in plasma protein percentage which would occur if 10 cc of protein free filtrate were removed from 100 cc of blood with a plasma volume of 50 per cent. The relative plasma volume would be decreased from 50/100, or 50 per cent, to 40/90, or 44.4 per cent—a reduction amounting to 11 per cent of the original figure. The plasma proteins, however, would be concentrated in proportion to the change in absolute plasma volume. Thus, 50 cc. of plasma would be concentrated to 40 cc. and, without addition of protein, the plasma proteins would be increased by 25 per cent. Therefore, the observation that plasma volume decreases less than the protein content increases does not indicate that protein has been added to the blood.

The data reported by Plass and Rourke have been collected in Table 2 and recalculated. It may be noted that in every instance but one (Experiment 9) the calculated total protein percentage is significantly greater than the observed value, indicating loss of protein from the blood stream. At a venous pressure of 100 mm Hg the filtrate contained between 2.5 and 3.1 per cent protein. At a venous pressure of 90 mm Hg the filtrate in one instance apparently contained no protein, while in the other two instances the filtrate contained 2.5 and 2.2 per cent of protein. In one observation at 80 mm Hg the filtrate contained 1.6 per cent of protein.

One may conclude therefore that at venous pressures of 80 mm Hg or more the capillary wall becomes relatively permeable to protein. At a venous pressure of 60 mm Hg conspicuous loss of protein could not be detected. The capillary filtrate appeared in four instances to contain 0.7 per cent protein or less.

TABLE 2  
Data from Plass and Rourke (1927, page 737)

Experiment number	Venous congestion		Plasma volume	Class of data	Albumin	Globulin	Fibrinogen	Total protein	Water loss	Total protein in filtrate
	Pressure	Duration								
	mm Hg	minutes	per cent		grams per 100 cc	grams per 100 cc	grams per 100 cc	grams per 100 cc	cc per 100 cc	grams per 100 cc
7	0		62.3	initial	4.85	2.50	0.24	7.59		
	100	15	53.5	observed	6.88	2.58	0.33	9.79		
				calculated	6.96	3.59	0.34	10.89	18.9	2.5
8	0		61.1	initial	4.76	2.47	0.29	7.52		
	100	20	45.0	observed	7.70	3.47	0.45	11.62		
				calculated	9.15	4.74	0.56	14.45	29.3	3.1
10	0		57.8	initial	4.45	2.27		6.72		
	100	22	44.3	observed	6.48	3.10		9.58		
				calculated	7.66	3.90		11.56	24.2	2.8
4	0		62.3	initial	5.02	2.27	0.29	7.58		
	90	15	56.9	observed	5.79	2.70	0.38	8.87		
				calculated	6.28	2.84	0.36	9.48	12.5	2.5
5	0		55.1	initial	4.70	2.16	0.26	7.12		
	90	15	46.9	observed	6.09	2.60	0.34	9.03		
				calculated	6.52	3.00	0.36	9.88	15.4	2.2
9	0		55.6	initial	5.00	2.31	0.29	7.60		
	90	21	48.1	observed	6.76	3.57	0.37	10.70		
				calculated	6.76	3.13	0.39	10.28	14.5	0.0
11	0		65.0	initial	5.25	2.51		7.76		
	80	26	62.0	observed	5.66	2.94		8.60		
				calculated	5.98	2.86		8.84	7.9	1.6

(C) *The relative increase in globulin and albumin percentages produced by high grades of venous congestion*

Since the globulin molecule is larger than the albumin molecule, venous congestion might be expected to increase the former fraction more than the latter. Table 3 shows a comparison of the percentage increase in the albumin and globulin (plus fibrinogen) fractions found in those experiments in which protein percentage changes were large enough to make the figures significant. In two instances, globulin (plus fibrinogen) was increased by a slightly greater amount than albumin, while in the other two instances the increases were about equal.

Plass and Rourke (1927) as well as Rowe (1915) found little regularity in the percentage increase of the various protein fractions of the plasma. In Table 2 the recalculated data of Plass and Rourke show in general

that fibrinogen, albumin and globulin have been lost in the capillary filtrate. The calculated values for albumin exceed the observed values in every instance but one. In the case of globulin the calculated values exceed the observed in 4 of the 7 experiments. In the remainder the

TABLE 3  
*Experimental data on protein fractionation*

Experiment number	Congestion pressure	Globulin	Albumin	Percentage increase	
				Globulin	Albumin
	mm. Hg	grams per 100 cc.	grams per 100 cc.	per cent	per cent
2	9	1.55	4.06		
	80	2.15	5.20	38.7	27.9
3	9	2.25	4.24		
	80	2.79	5.36	24.0	26.4
4	9	2.43	4.15		
	80	3.02	4.79	24.3	15.4
16	9	2.77	4.04		
	80	3.70	5.46	33.7	35.1

observed value exceeds the calculated globulin percentage. In the case of fibrinogen the calculated and observed values agree surprisingly well in four of the five experiments. In one experiment the difference amounts to .09 per cent.

(D) *The protein content of edema fluid accumulating in the tissue spaces during venous congestion*

In order to amplify the observations on composition of the capillary filtrate produced during mild venous stasis, fluid was removed from the lower extremities of two patients who were suffering from edema. In Case 1, venous pressure in one leg had been raised to 30 mm Hg for a period of several days by an intra abdominal metastatic neoplasm. Case 2 was suffering from nephrosis with generalized edema. The edema fluids in both instances contained relatively small amounts of protein.

Case 1, J. N., male, aged 46, was admitted to the University Hospital on September 11, 1931, complaining of weakness, nausea and anorexia. Examination showed a normal blood pressure, a marked secondary anemia, achlorhydria, a large epigastric mass and occult blood in the stools. The diagnosis of carcinoma of the stomach was verified by x ray and at laparotomy numerous metastases were found in the liver. He was discharged but returned to the hospital in December, 1931, with profound anemia and a still larger epigastric mass. During the two periods spent in the hospital five transfusions,



each of 500 cc of citrated blood, were given. On December 21, following the last transfusion slight edema of both ankles appeared. There was no evidence of cardiac or renal disease. The plasma protein percentage was still normal, 6.64 per cent, owing, no doubt, to the transfusions which had prevented the decrease usual in the malnutrition of gastric neoplasm. The osmotic pressure of the plasma proteins was measured and found to be 34.0 cm. water.

Seven days after the onset of the slight bilateral edema the fluid accumulation in the left leg increased rapidly, extending finally to the genitalia. At this time the patient complained of pain in the left calf. On examination the veins of the left leg were slightly but definitely engorged and the skin of the left foot was somewhat cyanotic in tint. Venous pressure was measured in a vein over the patella of each leg by means of a celluloid capsule (Krogh, Turner and Landis (1932)). In the recumbent position the venous pressure in the conspicuously edematous left leg was 30 mm. Hg, in the very slightly edematous right leg, 10 mm. Hg. Both legs were exposed to the air (temperature 24.0° C.) and skin temperature was measured over the instep by means of a thermal junction. The temperature of the left leg fell more rapidly than that of the right and remained finally 2.4 degrees cooler, indicating diminished circulation on the left side.

A single Southey's tube was inserted into the external aspect of the left leg above the ankle and approximately 40 cc of edema fluid were removed. The fluid was clear, slightly yellowish in color and did not coagulate. The first few cc of edema fluid contained 0.39 per cent of protein. A second determination, made on the fluid obtained about one hour later, showed the same protein percentage.

Case 2, E. McD., male, aged 21, was admitted to the University Hospital for the first time in May, 1929, suffering from a bloody diarrhea which had existed intermittently for ten years. The diagnosis of chronic ulcerative colitis was made at this time. He was treated in the hospital for six weeks with improvement. Following discharge he had alternating periods of improvement and regression, finally returning to the hospital November 10, 1931, complaining chiefly of "dropsy" and fatigue. On examination the patient showed marked pitting edema of both legs, ascites and right pleural effusion. The blood pressure was 140/80 and the urine contained on numerous occasions a cloud of albumin with many hyaline casts. Doubly refractile lipid bodies were present. The serum proteins were 3.56 per cent and the albumin-globulin ratio was 0.86. Blood cholesterol was 333 mgm. per 100 cc. The diagnosis at this time was chronic ulcerative colitis, lipid nephrosis. The edema proved resistant to the usual diuretic therapy which was handicapped by the patient's gastro-intestinal symptoms. It was decided to use Southey's tubes.

Six hours prior to their insertion the patient was seated in a chair with legs dependent. In this position the hydrostatic pressure of the column of blood in the veins amounted to 40 mm. Hg. The edema of the legs, moderate in the recumbent position, increased rapidly, with the change in posture. In the course of 30 hours, 6,500 cc of fluid were withdrawn. The protein content of this fluid was 0.09 per cent while the plasma proteins examined simultaneously were 3.27 per cent.

#### DISCUSSION

It was the purpose of these studies to measure by another method the effect of graded venous congestion on the movement of fluid from the blood to the tissue spaces. In contrast to the earlier findings of

Mende (1919), plethysmographic studies (Krogh, Landis and Turner (1932)) indicated that a relatively small rise in venous pressure was sufficient to cause small, but definitely measurable, amounts of fluid to accumulate in the tissues. This conclusion has been verified by the present studies, in that a venous pressure of 20 mm. Hg was accompanied in two instances by a slight but measurable loss of fluid from the blood stream.

The plethysmographic and the blood studies agree also in indicating an increasing rate of fluid filtration with increase in venous pressure. A straight line relationship was observed between venous pressure and filtration rate when the latter was measured by plethysmograph during venous congestion of 11 to 39 mm Hg (15 to 50 cm water). This relationship is not found when fluid loss from the blood is charted against the higher venous pressures used in the present studies. Blood flow, however, will be decreased particularly with the higher grades of venous congestion. Filtration at a given rate will concentrate the blood more when the rate of flow is simultaneously decreased. This factor was not of great importance in the plethysmographic studies in which the fluid filtration itself was measured at relatively low pressures. With venous pressures nearer diastolic arterial pressure the diminution of the blood flow is quite considerable (G N Stewart (1912-13)) and this must increase to some extent the loss of fluid from any given volume of blood. Moreover, with the highest venous congestion (80 mm Hg) there appears to be a measurable loss of protein through the capillary wall which must also facilitate the loss of fluid from the blood.

In explaining the movement of fluid through the capillary wall into the tissue spaces, the capillary endothelium has been regarded as relatively impermeable to the plasma proteins. Drinker and his co workers have called attention to the high protein content of lymph which may rise to 4.5 per cent (Drinker and Field (1931)). Loewen, Field and Drinker (1931) conclude that the effective osmotic pressure for returning water to the blood capillaries is the difference between the colloid osmotic pressure of the blood and the lymph from the area under observation. Yet during the venous obstruction produced by tying all the veins of an extremity of a dog (Field and Drinker, 1931) the amount of lymph produced was increased while the protein content diminished. In plasmapheresis experiments also the more rapid flow of lymph was accompanied by a smaller protein content. Attention has been called (Krogh, Landis and Turner (1932)) to the probability that the protein in lymph varies widely depending, among other things, on the rate of filtration and the relative amount of absorption. The slower the filtration and the more complete the reabsorption, the more will the composition of lymph differ from that of the capillary filtrate and hence from that of average tissue fluid. The more rapid the filtration (as in venous congestion and in plasmapheresis) the more nearly will both average tissue fluid

and lymph resemble the fluid filtered through the capillary wall. The fact that the more rapid lymph production in venous congestion was accompanied by decrease in protein content indicates that the previously existing difference between capillary filtrate and lymph was being reduced. It does not seem justifiable to state that the effective osmotic pressure for returning water to the blood capillaries is the difference between the colloid osmotic pressure of the blood and the lymph from the area under observation. Actually, lymph represents tissue fluid after the absorption process has been carried to as complete a stage as conditions in the blood stream permit.

Field and Drinker (1931) found that when all the veins to a limb were ligated the protein content of lymph did not fall below 1 per cent. The stasis produced by this procedure is of extreme grade, and is apparently accompanied by the passage of protein and red cells through the capillary wall.

When the effects of graded degrees of venous congestion are studied in man a loss of protein through the capillary wall is easily detected with venous pressures of 80, 90 and 100 mm Hg. The suggestion of Plass and Rourke that protein under these conditions moved from the tissue cells, to lymph and then into the blood stream has already been discussed.

At congestion pressures of 60 mm Hg the loss of protein was very much less and, except in one instance, too small to be detected in plasma protein analyses. It is believed that the findings at 60 mm Hg are significant but it is obvious that at 40 mm Hg the changes in blood volume are too small to permit any conclusion concerning the protein content of the capillary filtrate.

The tissue fluid obtained from two cases of edema during venous stasis indicates that the protein in the capillary filtrate (0.39 and 0.09 per cent) may be very much less than the protein content usually described for normal lymph. It is, therefore, not justifiable to assume that lymph represents the capillary filtrate nor even average tissue fluid unless absorption is completely avoided.

Nothing can be said concerning the mechanism by which the protein passes through the capillary wall at high congestion pressures. The presence of red cells in lymph (Field and Drinker) and petechiae frequently noted in the skin (Plass and Rourke) suggest mechanical rupture of the capillary wall. The fact that lymph protein in general decreases during such congestion in spite of the presence of red cells does not rule out mechanical rupture, since the cells and plasma escaping through a few weak places in the wall may be diluted by a relatively protein free filtrate from the remainder of the capillary. Anoxemia, which has been observed to change capillary permeability (Landis, (1928)), may also be a factor at high venous pressures.

The findings indicate that the permeability of the capillary wall with respect to protein varies with the grade of venous congestion. Caution must be used in extending conclusions based on observations involving high grades of venous congestion to considerations of normal capillary permeability.

#### SUMMARY

Blood samples removed from the arm veins during graded congestion were compared in order to measure the filtration of fluid resulting from the increased venous pressure. The loss of protein through the capillary wall was estimated at venous pressures of 80, 60 and 40 mm Hg.

Comparison of hemoglobin readings and red cell counts showed that during venous congestion the fluid is lost chiefly from the plasma.

The loss of fluid could be detected at venous pressures as low as 20 mm Hg and amounted to between 0.0 and 2.3 cc. per 100 cc. of blood.

The amount of fluid lost from the blood was conspicuously greater at higher venous pressures, venous congestion of 80 mm Hg filtered as much as 19.5 cc. per 100 cc. of blood.

At a venous pressure of 80 mm Hg protein was lost from the blood plasma in an amount indicating that the capillary filtrate contained an average of 1.5 per cent of protein. At a venous pressure of 60 mm Hg very little protein loss could be detected and the capillary filtrate contained an average of 0.3 per cent protein.

Two cases of edema are described in which edema fluid was collected during venous congestion. The protein content of the edema fluid was 0.39 and 0.09 per cent, indicating that the capillary wall retained approximately 95 per cent of the plasma protein.

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lins Troensegaard and Koudahl (11) were unable to bring down the cholesterol and phosphatides with the globulins if they were precipitated before the albumins

Hardy and Mrs Gardner (12), Chick (13), and Haslam (7) found phospholipid material associated with the globulin fraction of the plasma Macheboeuf (14) precipitated a protein fraction from horse serum and plasma with ammonium sulfate which upon reprecipitation was constant in composition It contained 22.7 per cent phosphamino lipids, 17.9 per cent cholesterol esters, and 59.1 per cent proteins This is the largest amount of total lipid associated with protein precipitates reported Macheboeuf, Wahl, and Sandor (15) also reported analyses on normal human plasma in which they found that the free cholesterol exceeded the cholesterol esters associated with the albumins, and that the reverse was true in the case of the globulins

Lustig and Katz (16) studied both the phospholipid and cholesterol content of beef plasma proteins They found less cholesterol and phospholipids in the euglobulin fraction than in the pseudoglobulin and albumin fractions Went and Goreczky (17) showed that when serum proteins underwent an ultrafiltration process the cholesterol and phospholipid in the filtrate varied directly with the amount of protein present A lecitho-protein present in tissue fibrinogen was described by Mills (18) This combination, however, presents a problem of different character from that found in the case of globulins and albumins

The possibility of interpreting abnormal lipid-protein values under pathological or experimental conditions, and also of obtaining information upon the type of association present led the authors to a detailed study of the problem Since abnormal conditions were to be studied on both animal and human blood, it was thought desirable to make a preliminary study of the normal relationships between species For this purpose three types of blood, namely, human, dog, and horse, were employed, and a study of the lipids associated with the albumin and globulin fractions was made

#### METHODS

*Precipitation of the proteins* A portion of plasma or serum (at least 10 cc) was diluted with three volumes of water and brought to one-fourth saturation with ammonium sulfate solution at room temperature If a precipitate of fibrinogen formed, it was removed and discarded The globulins were then precipitated by half saturation and removed from the solution by centrifugation Filtering in the usual manner was avoided in order to prevent loss of lipid material on the filter paper The precipitated protein was washed with half-saturated ammonium sulfate solution and the washings were added to the solution containing the albumins The globulins were dissolved in water Both fractions were coagulated by heat and the pH was adjusted to the point of maximum precipitation by the addition of 10 per cent acetic acid After standing until the precipitates settled, the supernatant liquids were removed by siphoning Several washings were made with distilled water in order to

remove the salt from the proteins. No attempt was made to remove the last traces of ammonium sulfate as it did not interfere with the extraction of the lipids.

*Extraction of the lipids* The lipids were removed from the moist proteins by extracting with hot 95 per cent alcohol three times, hot absolute alcohol once, and ether three times, all in the same tube. After centrifugation, the solvents were decanted while still warm. The amounts of solvents were in such proportion as to give when mixed a solution containing approximately 35 per cent ether and 65 per cent alcohol. After cooling, the extracts were diluted to a definite volume. The albumin fraction required less intensive extraction for removal of the lipid material than did the globulin fraction. This method of extraction was adapted from Cavett (19) who found that the proteins extracted by this method were completely freed from lipids. He also demonstrated the importance of removing the lipids from the proteins while they were yet moist.

A second portion of plasma or serum, usually consisting of 5 cc., was extracted with 100 cc. of hot alcohol-ether solution by the regular Bloor procedure (20). The statement of Bloor that these proteins were free from lipids was also verified by Cavett (19).

The extracted proteins were dried and weighed. When the amount of plasma permitted, the relative amounts of the proteins were also determined colorimetrically by a modification of the Wu method (21).

It was first planned to fractionate and study the euglobulins since some previous workers included this protein in their studies. However, they worked with large amounts of plasma and in human cases this was not practicable. In two cases, however, the euglobulin, pseudoglobulin and albumin were fractionally precipitated from diluted plasma by means of ammonium sulfate. It was obvious that the amount of pseudoglobulin in normal plasma was so small that a volume of blood approximating 50 cc. would have been necessary in order to obtain a sufficient quantity for reliable analysis of the extracted lipids. About one-third of the globulins is composed of pseudoglobulin or less than one gram per 100 cc. of plasma. For this reason, it was decided to deal only with total globulins and albumins. Information obtained subsequently on pathological material (22) strengthened this decision as the euglobulin fraction became very small with a corresponding increase in the pseudoglobulin fraction.

*Determinations of lipids* Bloor's oxidative method (23) was used for the determination of fatty acids. This method consists essentially of saponification of the alcohol-ether solution, extraction of the acidified residue with petroleum ether, and oxidation of the ether residue with sulfuric acid dichromate reagent. Correction was made for the cholesterol content.

The phospholipids were determined as lecithin phosphorus according to the method of Bloor (24) and Benedict and Theis (25). According to this procedure the extracted lipids are oxidized by a mixture of nitric and sulfuric acids and the phosphoric acid reduced by hydroquinone-sulfite and sodium molybdate to a blue compound which is estimated colorimetrically.

The free and total cholesterol were analyzed by Turner's (26) modification of the Okey method (27) for the determination of cholesterol by oxidation of the digitonide. Iodine numbers were determined by Gibson's micro method (28).

## RESULTS

The alcohol-ether extract of the protein fractions and also of the total plasma were examined for their content of fatty acids, lecithin, and free

and total cholesterol. Iodine numbers of the total lipids were determined. Results are expressed as percentage of lipid per hundred cc of plasma and also as mgm of lipid per gram of protein since the amounts of globulin and albumin per unit volume are not the same. Typical determinations on human and dog plasma and horse serum are shown in Tables I, II, and III, respectively. The range of results between samples is shown in Tables IV and V, in which the maximum and minimum lipid values obtained from four samples of plasma are given. Vertical results do not necessarily refer to the same sample. The relative amounts of lipids in the globulin and albumin fractions, expressed as ratios, are given in column 4 in each table.

The data on human blood came from four normal healthy individuals, who were bled usually at 11 a.m. The precipitated plasma proteins contained about 50 per cent of the total lipids present in the plasma, although there was great variation when the individual lipids were considered (Table I). Approximately 45 per cent of the total fatty acids, 28 per cent of the phospholipids and 75 per cent of the total cholesterol were found in the protein fractions. A larger percentage of free cholesterol than of cholesterol esters was removed with the proteins, however, since the free cholesterol constituted less than half of the total, the absolute amount of cholesterol esters found with the proteins was larger.

Per unit weight, the globulin fraction contained more lipid material than the albumin fraction. Average figures for the total lipids showed that the globulins contained about one-third more lipid material than the albumins per gram of protein. The ratio of globulin lipid to albumin lipid was greatest for phospholipid and least for cholesterol. However, when the lipids were calculated as mgm per 100 cc of plasma, the albumin fraction contained a higher percentage than the globulin fraction since there was more albumin than globulin. Per volume of plasma there was approximately twice as much total cholesterol associated with the albumin fraction as with the globulin fraction. However, the globulins constituted about one-third of the total proteins present, so that there was little difference in the distribution of cholesterol per gram of protein between the two fractions. About 45 per cent of the fatty acids and also of the phospholipids were found with the globulins. The globulin fraction both by weight and volume included a larger percentage of the ester than of the free cholesterol. There was a greater variation between samples in the cholesterol content of the proteins per unit of volume than in the fatty acid or phospholipid content (Table IV).

The iodine numbers of the total lipids brought down with the globulin and albumin fractions were lower than those of the total plasma lipids. A slightly lower iodine number was always found on the lipids from the albumin fraction than from the globulin lipids. These facts suggest that some oxidation of the lipids might have taken place during the preparation of the protein fractions.

TABLE I  
Normal human plasma (one sample)

Class of lipid	Globulin lipid mgm.	Globulin lipid Total protein lipid per cent	Albumin lipid mgm.	Globulin lipid Albumin lipid ratio	Total pro- tein lipid (calculated) mgm.	Protein lipid Total lipid per cent	Total plasma lipids mgm.
Fatty acids * per 100 cc plasma	100.0	43.1	132.0		232.0	45.5	510.0
per 1 gram respective proteins	35.8		24.0	1.49			
Lecithin per 100 cc plasma	30.0	45.5	36.0		66.0	27.8	238.0
per 1 gram respective proteins	10.7		6.5	1.63			
Cholesterol							
Free per 100 cc plasma	26.0	34.2	50.0		76.0	78.4	97.0
per 1 gram respective proteins	9.3		9.1	1.02			
Ester per 100 cc plasma	32.0	36.4	56.0		88.0	74.5	118.0
per 1 gram respective proteins	11.4		10.2	1.12			
Total per 100 cc plasma	58.0	35.4	106.0		164.0	76.3	215.0
per 1 gram respective proteins	20.7		19.2	1.08			
Total lipid per 100 cc plasma	158.0	40.0	238.0		396.0	54.6	725.0
per 1 gram respective proteins	56.4		43.3	1.30			
Iodine number	64		57				75

Protein content by weight Albumin 5.5 grams per 100 cc.

Globulin 2.8 grams per 100 cc.

Total 8.3 grams per 100 cc.

\* Corrected for cholesterol



TABLE II  
Normal dog plasma (one sample)

Class of lipid	Globulin lipid mgm	Globulin lipid Total protein lipid per cent	Albumin lipid mgm	Globulin lipid Albumin lipid ratio	Total pro- tein lipid (calculated) mgm	Protein lipid Total lipid per cent	Total plasma lipids mgm
Fatty acids * per 100 cc plasma	77.0	57.0	58.0		135.0	42.1	321.0
per 1 gram respective protein	30.0		15.9	1.88			
Yeastin per 100 cc plasma	26.0	61.2	16.5	2.24	42.5	32.0	132.0
per 1 gram respective protein	10.1		4.5				
Cholesterol							
Free per 100 cc plasma	16.1	43.4	21.0		37.1	71.1	50.0
per 1 gram respective protein	6.3		5.7	1.10			
Ester per 100 cc plasma	21.0	46.7	24.0		45.0	67.2	67.0
per 1 gram respective protein	8.2		6.6	1.24			
Total per 100 cc plasma	37.0	44.6	46.0	1.15	83.0	71.0	117.0
per 1 gram respective protein	14.5		12.6				
Total lipid per 100 cc plasma	114.0	52.3	104.0	1.56	218.0	49.8	438.0
per 1 gram respective protein	44.5		28.5				
Iodine number	72		60				81

Protein content by weight Albumin 3.65 grams per 100 cc

Globulin 2.56 grams per 100 cc

Total 6.21 grams per 100 cc

\* Corrected for cholesterol

TABLE III  
Normal horse serum (one sample)

Class of lipid	Globulin lipid mgm.	Globulin lipid Total protein lipid per cent	Albumin lipid mgm	Globulin lipid Albumin lipid ratio	Total pro- tein lipid (calculated) mgm.	Protein lipid Total lipid per cent	Total serum lipids mgm.
Fatty acids * per 100 cc serum	132.0	75.0	44.0		176.0	44.0	400.0
per 1 gram respective protein	28.7		20.0	1.43			
Lecithin per 100 cc serum	68.0	77.4	20.0		88.0	33.8	260.0
per 1 gram respective protein	14.8		9.1	1.63			
Cholesterol							
Free per 100 cc serum	36.8	68.2	17.2		54.0	72.0	75.0
per 1 gram respective protein	8.0		7.8	1.03			
Ester per 100 cc serum	35.8	70.2	15.2		51.0	63.7	80.0
per 1 gram respective protein	7.8		6.9	1.13			
Total per 100 cc. serum	72.6	69.2	32.4		105.0	67.7	155.0
per 1 gram respective protein	15.8		14.6	1.08			
Total lipid per 100 cc serum	204.6	73.0	76.4		281.0	50.6	555.0
per 1 gram respective protein	44.6		34.7	1.29			
Iodine number	65		54				77

Protein content by weight Albumin 2.2 grams per 100 cc.  
Globulin 4.6 grams per 100 cc.

Total 6.8 grams per 100 cc

\* Corrected for cholesterol

TABLE IV  
Normal human variations (four samples of plasma)

Class of lipid	Globulin lipid mgm	Globulin lipid Total pro- tein lipid per cent	Albumin lipid mgm	Globulin lipid Albumin lipid ratio	Total pro- tein lipid (calculated) mgm	Protein lipid Total lipid per cent	Total plasma lipids mgm
Fatty acids * per 100 cc plasma per 1 gram protein	100.0-125.0 35.8-38.1	42.0-46.0	125.0-110.0 23.0-27.1	1.38-1.49	225.0-265.0	11.0-18.0	150.0-555.0
lecithin per 100 cc plasma per 1 gram protein	30.0-36.5 9.2-11.3	44.0-49.0	36.0-44.5 6.5-8.7	1.63-1.85	66.0-81.0	28.0-35.0	210.0-238.0
Cholesterol							
Free per 100 cc plasma per 1 gram protein	14.1-31.2 5.0-9.5	30.0-41.0	25.9-50.0 4.7-9.1	1.02-1.15	40.0-76.0	70.0-78.1	67.5-97.0
Ester per 100 cc plasma per 1 gram protein	25.0-36.0 9.2-11.4	32.0-40.0	48.0-56.0 8.7-10.3	1.12-1.21	73.0-90.0	65.0-71.5	112.0-123.0
Total per 100 cc plasma per 1 gram protein	39.1-67.2 14.0-20.7	34.0-42.0	73.9-106.8 13.4-19.2	1.04-1.09	115.0-161.0	64.0-76.3	179.5-220.0
Total lipid per 100 cc plasma per 1 gram protein	139.1-192.2 49.5-59.0	40.0-45.0	198.9-246.0 36.2-46.0	1.26-1.40	338.0-438.0	48.0-54.6	629.5-775.0
Iodine number	51-66		44-60				61-77

Protein content by weight Albumin 4.10-5.50 grams per 100 cc  
 Globulin 2.10-3.28 grams per 100 cc  
 Total 6.20-8.30 grams per 100 cc

\* Corrected for cholesterol

TABLE V  
Normal dog variations (four samples of plasma)

Class of lipid	Globulin lipid mgm.	Globulin lipid Total pro- tein lipid	Albumin lipid mgm.	Globulin lipid Albumin lipid	Total pro- tein lipid (calculated)	Protein lipid Total lipid per cent	Total plasma lipids mgm.
Lipids acids * per 100 cc. plasma	67.2-77.0	51.0-57.0	58.0-68.0	ratio	132.0-142.0	42.1-47.0	280.0-340.0
per 1 gram protein	29.6-32.0		15.9-17.0	1.74-1.88			
Lecithin per 100 cc. plasma	21.0-26.0	57.0-61.0	16.0-18.0	2.10-2.40	37.0-44.0	30.0-37.0	100.0-140.0
per 1 gram protein	9.6-10.1		4.2-4.5				
Cholesterol							
Total per 100 cc. plasma	27.0-40.0	59.0-65.5	42.0-55.0	1.15-1.26	69.0-95.0	61.0-74.0	103.0-128.0
per 1 gram protein	12.7-16.0		11.1-13.8				
Total lipid per 100 cc. plasma	94.2-117.0	50.5-52.3	100.0-123.0	1.52-1.70	201.0-237.0	48.0-52.5	283.0-468.0
per 1 gram protein	44.9-47.0		29.0-30.8				
Iodine number	60-72		52-60				68-81

Protein content by weight Albumin 3.65-4.00 grams per 100 cc.  
 Globulin 2.10-2.56 grams per 100 cc.  
 Total 5.80-6.56 grams per 100 cc

\* Corrected for cholesterol

Four samples of fasting dog plasma were obtained from three animals weighing between 10 and 13 kilograms, all on the same diet. The lipid content was lower than that of human plasma. Because of the small amount of cholesterol present, it was difficult to obtain both free and total cholesterol, but in one case enough blood was drawn for the complete analysis (Table II). The plasma proteins carried down about half the lipid material, a finding corresponding to the results obtained on human plasma. The percentages of individual lipids included with the protein fractions were also similar. However, the globulin fraction per gram of protein as well as per unit of volume contained more lipid material than was found in the human plasma. The increase per unit volume was about 10 per cent for each lipid. The ratio between the amounts of phospholipid per gram of globulin and of albumin was above two. Fatty acids showed a corresponding increase and cholesterol increased to a lesser degree. The relationships between free and total cholesterol and the protein fractions were not essentially altered. The variations between samples (Table V) were greater in the cholesterol content of the proteins than in the fatty acid or phospholipid contents, as was true in the human plasma. The iodine numbers also maintained the same relations to the total plasma as in human blood.

The horse serum<sup>1</sup> contained larger amounts of globulins than of albumins in the two samples studied. The analysis obtained on one sample is given in Table III. The variations between the two samples were slight. Since the cholesterol content of horse serum is low, sufficiently large quantities were used to determine both free and total cholesterol. The amounts of fatty acids and of phospholipid were greater than those of dog plasma. Approximately the same percentage of lipid material was found with the total protein fractions as well as the same proportions of the individual lipids, as in the human and dog samples. The greater percentage of free cholesterol was also found with the proteins as in the previous determinations. However, since the free cholesterol constituted nearly 50 per cent of the total cholesterol, the total proteins contained nearly equivalent amounts of the two forms of the sterol. This is not a significant variation since the normal proportion of free to total cholesterol is not a constant figure. In the globulin fraction the amount of lipid material per unit volume was increased by an average of 30 per cent over that found in the other two types of blood. However, there was no marked increase in the globulin-albumin lipid ratio per gram of protein as compared to the results obtained from human plasma, because of the greater globulin content. Per unit weight, the globulins contained about one-third more lipids than the albumins. The globulin-albumin phospholipid ratio exceeded the fatty acid or cholesterol

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<sup>1</sup> We are indebted to Parke Davis and Company for a generous supply of normal horse serum.

ratios. Slightly less cholesterol ester than free cholesterol was found on the globulin fraction, a difference which, however, is not a significant variation from the condition present in the human or dog plasma. The iodine numbers were lower for the protein lipids than for those of the whole plasma, and also lower for the globulin lipids than for the albumin lipids.

#### SUMMARY AND CONCLUSIONS

1 The albumins and globulins have been fractionated by means of salt precipitation from human and dog plasma and horse serum. Fatty acids, phospholipids, and cholesterol have been determined in these protein fractions.

2 In general, the total proteins carried down about one half of the lipid content of the plasma or serum. The globulins showed a definite tendency to contain more lipids than did the albumins. They also contained varying amounts of the individual lipids, the phospholipids being present in a greater percentage than either fatty acids or cholesterol.

3 In horse serum, in which the amount of globulins was larger than of albumins there was no marked change in the amount of lipid per gram of protein, although such was the case per volume of serum.

4 The globulins of the dog plasma contained significantly more of all three lipids than did those of horse serum or human plasma, the increase being the greatest in the case of the phospholipids.

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## THE BLOOD URIC ACID IN DISEASE

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Because of the large number of patients in the University of Chicago Clinics showing high blood uric acid by the Koch<sup>1</sup> modification of the Folin and Benedict methods using the Folin-Wu blood filtrate, it was decided to try a different method. The method which has seemed to yield most satisfactory results is that described by Folin (1) in which he makes use of unlaked blood filtrate, a cyanide solution stabilized by means of urea, and a uric acid reagent free from phenol reagent. This method has been used for all uric acid determinations in the Medical department since May 1931, and has proved relatively satisfactory as a clinical method. The cyanide solution, which is kept at low temperature, retains its chromophoric power well, and when used with the uric acid reagent has produced no turbid solutions. It was, of course, necessary to determine a new series of normal blood uric acid values by this method, and while the results are not wholly free from unexplained high values (see Table V), it is believed that this method, as compared with the one formerly used, gives more satisfactory results.

For the purpose of comparison a few samples of blood were tested simultaneously for uric acid by the Koch modification formerly used (hereafter called "old method") and by the Folin method at present used. Two samples from a control patient, drawn on different days, gave 7.2 and 9.2 mgm. per 100 cc. by the old method and 3.9 and 3.8 mgm. per 100 cc. respectively by the new method. One sample from a patient with chronic gout gave 12.3 and 5.6 mgm. per 100 cc. by the old and new methods respectively. The uric acid in the blood of a patient with infectious arthritis was 5.3 and 2.1 mgm. per 100 cc. by the old and new methods respectively. There was thus a deviation in all instances in the same direction and to about the same degree, but there also seemed to be less variation in the results given by the method at present in use.

The first and most important consideration for diagnostic purposes in adopting a new method of determining the amount of uric acid in the venous blood was to find an upper limit for normal which would be reasonably satisfactory.

In Table I are a number of uric acid determinations made on disease-free controls. It will be seen that in only two, Case 6, W. C., and Case

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<sup>1</sup> Koch, F. C. Modification developed at University of Chicago



TABLE I  
Disease-free controls

Case		Age	Uric acid in mgm. per 100 cc					
		Years						
1	L. C.	30	2.8	3.0				
2	J. G.	30	3.0	2.5				
3	M. H.	31	2.5	2.4				
4	H. H.	28	2.5	2.2				
5	R. H.	39	2.8	3.2				
6	W. C.	43	5.1	4.0	4.3			
7	B. I.	30	3.6	3.2				
8	H. P.	28	3.5	3.4				
9	E. J.	29	3.9	3.8	3.2	3.4	4.8	3.6
10	R. P.	27	3.4					
11	D. G.	27	3.2	2.6				
12	H. A.	36	3.3	2.4				
13	C. M.	41	2.6	3.0				

9, E. J., were any results found higher than 4 mgm. per cent. In one of these (Case 9) the high value obtained was only one of six, the others being normal, and this was interpreted as being of no significance. The other (Case 6) in which the uric acid value remained persistently above 4 mgm. was puzzling. In spite of the fact that this individual is apparently healthy in every way, and is one of the control cases, we are considering the uric acid values as abnormal.

In Table II are given a number of presumably normal uric acid readings and the clinical diagnosis in each case. It will be noted that in none of these is the uric acid above 4 mgm. per 100 cc. As a result this figure—4 mgm. of uric acid per 100 cc.—is considered to be the usual upper limit of normal, subject to the qualifications discussed later.

The problem which prompted our interest in this study was that of gout. Because of several rather dramatic episodes in the diagnosis of this condition it became desirable to determine by the new method the uric acid levels occurring in this disease. Table III gives the amount of uric acid obtained in patients with gout. It was at first intended to attempt the differentiation of acute gout and chronic gout, but because of the tendency for one to shade into the other and because of the complications resulting from diet and medication this was deemed impracticable. It is noteworthy that the uric acid level is in most instances considerably above the upper limit of those believed to be normal as shown in Tables I and II. In none of these patients was the blood uric acid the sole basis of diagnosis, though we have found it an important aid.

It is well recognized (Peters and Van Slyke (2)) that the blood uric acid may be raised above normal in a number of conditions other than true gout. It has been reported that it may or may not be raised in nephritis with impaired kidney function (3), and that has been our ex-

TABLE II  
 "Normal" blood uric acid

Case	Diagnosis	Age	Uric acid in mgm. per 100 cc.	
		years		
14 W T	Cholelithiasis, colitis	54	2.9	
15 J C	None made, neurologic?	19	3.4	
16 A B	Postoperative adhesions	69	3.4	
17 M B	None made	33	3.4	
18 F C	Colitis	45	1.6	
19 G M	Carcinoma of pancreas, chronic cystitis	76	2.2	
20 A C	Toxic liver necrosis	48	1.9	
21 V K.	Myasthenia gravis	24	3.0	
22 V D	Duodenal ulcer, colitis	48	3.0	
23 J S	Hypertension myocardial insufficiency	51	2.9	
24 S H	Maxillary sinusitis	45	2.9	
25 M O D	Carcinoma of thyroid	72	1.0	
26 W D	Malignant obstructive jaundice	57	1.5	
27 H B	None made	47	2.4	
28 L S	None made	34	3.8	
29 L G	Acute glomerulonephritis	24	3.7	
30 C M	Acute glomerulonephritis	24	2.2	
31 E M	Chronic glomerulonephritis	23	2.3	2.1
32 A. D	Proliferative arthritis	14	2.6	
33 W C	Proliferative arthritis	64	3.1	
34 S C	Proliferative arthritis	58	2.8	3.0
35 G S	Proliferative arthritis	48	1.1	
36 J A	Proliferative arthritis	58	2.5	
37 M A	Proliferative arthritis	66	3.3	
38 H D	Proliferative arthritis	26	1.9	1.3 2.3
39 F H	Proliferative arthritis	19	2.8	
40 J B	Proliferative arthritis	52	2.1	
41 H McC	Proliferative arthritis	35	3.3	
42 E D	Proliferative arthritis	56	1.7	
43 E O	Proliferative arthritis	31	2.6	
44 S B	Proliferative arthritis	44	3.2	
45 M H	Proliferative arthritis	32	3.7	
46 M K	Traumatic internal derangement of knee	26	2.6	
47 D R	Acute sacroiliac strain	32	2.8	
48 M B	Rheumatic fever?	21	2.1	
49 E. R.	Radiculitis	40	3.8	
50 E S	Rheumatic pains, colitis	47	2.2	
51 J B	Osteochondritis desicans	29	2.6	
52 A W	Degenerative arthritis diabetes	60	3.7	
53 K. J	Degenerative arthritis	70	2.9	
54 R S	Acute rheumatic fever	21	1.1	
55 E F	Degenerative arthritis	70	3.0	
56 M F	Degenerative arthritis	65	3.3	
57 M R	Degenerative arthritis myxedema	55	3.1	
58 N H	Acute myositis lues latens	50	3.2	
59 B J	Degenerative arthritis, lues latens	43	3.4	

TABLE III

*Gout*

Case	Age	Uric acid in mgm. per 100 cc.							
60 A	50	52	52	49	50	48	77	45	
61 J I	48	44							
62 A S	66	60	61						
63 H H	48	39	42						
64 C H	46	46							
65 I S	57	57	38						
66 D D	51	56	102	74					
67 C I	57	52	58	58	41	44	44	59	
68 J S	63	45	45	43	48	52	44	51	38
69 D I	62	42	43	39					
70 H H	68	71							
71 W K	50	54							
72 S L	45	49	47	36	43	50			
73 I W	48	83	49	52	61				
74 I B	43	47	50						
75 Z B	46	59							
76 H C	35	67	56	59					

perience In Table IV are given the results in a series of patients where the increase in blood uric acid may be explained on a basis other than gout In Case 85 where one determination was above the expected normal and the others were normal, the diagnosis was not clear and the possibility of visceral gout was considered Because of the first result the patient was placed on a purine-poor diet and was living on this regime when the other three determinations were made Many of the results given in Table IV are well within the range of gout, but two are higher than any we have yet found in gout

It remains to include in Table V those high uric acids which cannot be explained by any information available to us There have not been many of these If more tests had been made on these patients it is possible that some of the high values obtained would have been shown to be

TABLE V

*Unexplained high uric acids*

Case	Diagnosis	Age	Uric acid in mgm. per 100 cc.	
		years		
90 M H	Metatarsal fracture	26	50	
91 F L	Obesity, cholecystitis	50	41	
92 M M	Migraine ? headaches	48	41	
93 K M	Proliferative arthritis	59	44	37
94 G R	Sjögren's Disease (quiescent)	14	58	
95 N T	Proliferative arthritis	68	45	
96 A Z	Degenerative arthritis ?	51	41	
97 E L	Tuberculosis, mild diabetes	60	42	

TABLE IV  
*Increased blood uric acid other than gout*

Case	Diagnosis	Age years	Kidney function	Uric acid in mm. per 100 cc.		
77 F H	Chronic glomerulonephritis	48	Impaired	7	7	
78 G B	Pulmonary tuberculous arteriosclerosis	65	Impaired	5	6	
79 M G	Multiple myeloma	57	Not examined	5	0	
80 C V	Melanocarcinoma gallbladder	44	Normal	2	4	4 1
81 D J	Chronic myelogenous leukemia	45	Not examined	4	6	3 1
82 R G	Acute yellow atrophy Death	21	Normal (early)	2	2	4 4
83 W P	Chronic lymphatic leukemia	27	Not examined	5	0	
84 J C	Chronic glomerulonephritis	24	Impaired—uremia	16	2	
85 C C	No diagnosis made	42	Not examined	5	3	2 7
86 L W	Polycythemia vera	60	Not examined	4	0	3 4
87 M T	Arteriosclerotic heart disease	70	Impaired	12	6	
88 M B	Uremia			17	4	
89 A F	Lead poisoning ?	53	Not examined	3	88	4 1

negative results and of no diagnostic significance. They must, however, be included as indicating flaws in the test as a diagnostic procedure.

#### DISCUSSION

With the discovery of the uric acid diathesis in gout, attention was focused on purine metabolism. Further studies have indicated that the relationship between uric acid retention and gout is not as simple as at first believed. Observers have reported considerable variation in the amount of blood uric acid under normal conditions as well as in diseases other than gout. The significance of these findings is not as yet thoroughly understood. Although mention of gout in the contemporary American literature is unusual, it has been our experience to find the condition among Chicago patients with greater frequency than was anticipated. Whether a similar incidence of gout occurs in other parts of the country would be a matter of interest.

The amount of uric acid in the blood under normal and pathologic conditions is a matter of basic importance. The more accurately the determinations can be made the sooner will the understanding of purine metabolism approach scientific precision. By Folin's method, here in use, we believe that most, if not all, patients with gout will show blood uric acid values above a range which we accept as normal. The difficulty in diagnosis lies in distinguishing the higher values obtained in conditions other than gout. Most of such high values have occurred in the various conditions already known to be at times associated with high blood uric acid.

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# A STUDY OF THE GASTRIC SECRETION IN HYPERTHYROIDISM BEFORE AND AFTER OPERATION

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Gastric symptoms have long been associated with hyperthyroidism. The reports of studies attempting to correlate these symptoms with disturbances in gastric secretions are not uniform. Several investigators (1, 2) report an increase in the hydrochloric acid and total volume of secretion. One (3) contends there is no characteristic secretion in exophthalmic goiter. A greater number (4-16) report a diminution or absence of hydrochloric acid in hyperthyroidism and in the feeding of thyroid extracts to animals. Excellent reviews of the subject have appeared in recent papers by Moll and Flint (9), Brown (10), and Lerman and Means (13). With the development of a more accurate method for determining the degree of gastric acidity by histamine stimulation it seemed desirable to restudy the problem. Accordingly, a survey was begun eighteen months ago to determine the behavior of gastric secretion in hyperthyroidism before and after operation. Since the beginning of this work, Lerman and Means (13) have reported a study of gastric secretion in exophthalmic goiter before treatment using a similar method.

## METHOD

The present generally accepted technique of gastric analysis was employed in this study. A fasting specimen was withdrawn, followed by the ingestion of 50 cc. of 7 per cent alcohol. Specimens were removed at twenty and forty minute intervals. If the last specimen contained no hydrochloric acid, histamine (ergotamine acid phosphate) 0.1 mgm. per ten kilograms was given subcutaneously and specimens were withdrawn at twenty and forty minute intervals. The volume, free hydrochloric acid and total acidity of each specimen were determined. Töpfer's reagent and phenolphthalein respectively were used as indicators and the results expressed as the number of cc. of N/10 NaOH necessary to neutralize 100 cc. of gastric juice.

Two analyses were done on each patient before preoperative iodine therapy was begun, a third the day before operation, and a fourth the day of discharge. The patients were followed by subsequent analyses at intervals of one to two months. We have arbitrarily classified the results upon the basis of the highest acid content obtained after the stimulation by alcohol and histamine. (1) Normals, those patients in whom repeated analyses showed free hydrochloric

(1) Normoacidity—those with total acidity of 20 cc or above (2) Hypoacidity, achlorhydria—hydrochloric acid of 10 cc or less and total acidity below 20 cc (3) Achlorhydria—those with no hydrochloric acid 40 minutes after histamine stimulation

### MATERIAL

The gastric secretions of sixty-three patients with disease of the thyroid gland have been studied. Of these, nine had adenomatous goiters with normal function. One had an acute thyroiditis, also with normal function. Of the remaining fifty-three cases, thirty-five had exophthalmic goiter and fifteen had adenomas with elevated basal metabolism. The remaining three cases, which it seemed fair to exclude from this report, were complicated by conditions in which achlorhydria frequently occurs (pulmonary tuberculosis, diabetes mellitus, chronic alcoholism). The ages of the patients ranged from sixteen to seventy-two with three below twenty years and three above sixty years. There were forty-five females and fifteen males.

### RESULTS

#### *I Gastric secretion in disease of thyroid gland before treatment*

*A Adenomatous goiters with normal function* Control observation on the nine cases with adenomatous goiters with normal function and the case with acute thyroiditis showed normal gastric acidity and volume of secretion.

*B Hyperthyroidism* Of the thirty-five patients with exophthalmic goiter, twenty-four or sixty-nine per cent had an achlorhydria before preoperative iodine therapy, eight an hypoacidity, and three a normal secretion. In the group of fifteen adenomatous goiters with hyperfunction ten or sixty-seven per cent showed no hydrochloric acid and the remaining five had an hypoacidity. Thus, for the group of fifty patients with hyperthyroidism, thirty-four or sixty-eight per cent had an achlorhydria and thirteen or twenty-six per cent had an hypoacidity. The diminution in volume of gastric secretion paralleled the diminished acid content in the cases with hypoacidity and achlorhydria.

The results of this study have been analyzed to determine whether there was any correlation between the incidence of achlorhydria and the age of the patient, the duration of the disease, or the elevation of metabolism. From the data in Tables I and II we have concluded that age plays no significant part in the occurrence of achlorhydria in hyperthyroidism. While it is true that the incidence of achlorhydria by age decades seemed to increase up to forty-five years, on the other hand when patients are divided into those above and those below the age of forty, sixty-nine per cent of twenty-six patients above forty years and sixty-seven per cent of the twenty-four patients under forty showed an absence of hydrochloric acid. The observation that the age of the patients with

TABLE I

*Incidence of achlorhydria and reappearance of HCl by age decades*

Age decades	Number of cases	Gastric symptoms	Achlorhydria	Hypocidity	Normal	Hydrochloric acid returned	Hydrochloric acid not returned	Not followed	Average duration of symptoms	Basal metabolic rate before feeding	Per cent with achlorhydria	Per cent showing return of HCl
15-25	9	2	4	3	2	4	0	0	8 months	55	44	100
26-35	10	4	7	3	0	3	2	2	12 months	51	70	60
36-45	14	5	14	0	0	8	4	2	10 months	49	100	67
46-55	11	5	4	6	1	2	0	2	1 year 8 months	66	36	100
56-75	6	3	5	1	0	2	1	2	7 years	44	83	67
	50	19	34	13	3	19	7	8				

TABLE II

*Incidence of achlorhydria and reappearance of HCl by age decades*

Age groups	Number of cases	Achlorhydria	Hypochlorhydria	Normal	Hydrochloric acid returned	Hydrochloric acid not returned	Not followed	Per cent with achlorhydria	Per cent showing return of HCl	Remarks
Under 40	22	14	6	2	9	2	3	64	67	Exophthalmic goiters
Over 40	13	10	2	1	7	2	2	77	70	
Under 40	2	2	0	0	1	1	0	100	50	Adenomas with hyperfunction
Over 40	13	8	5	0	2	1	4	61.5	50	
Total for those under 40	24	16	6	2	10	3	3	67	76	Exophthalmic goiters and adenomas
Total for those over 40	26	18	7	1	9	7	5	69	69	

hyperfunctioning adenomas as a group was higher than that of those with exophthalmic goiter, with practically the same percentage of achlorhydria in each group, is further evidence in support of this conclusion

The duration of the hyperthyroidism does not appear to influence the incidence of achlorhydria. In Table III, it will be noted that sixty nine per cent of thirteen cases with symptoms for two years and over had achlorhydria, while sixty-two per cent of twenty six cases with symptoms of under one year's duration likewise had an absence of hydrochloric acid. Of the twenty four patients with a history of more than one year's duration, eighteen had achlorhydria as against sixteen of twenty-six patients with a duration of disease of less than one year.

A similar lack of correlation was found between the incidence of achlorhydria and the elevation of metabolism (see Table IV). Of the



TABLE III

*Percentage of achlorhydria in duration of the disease*

Duration of disease	Number of cases	Number of achlorhydria	Hypochlorhydria	Normal	Gastric symptoms	Hydrochloric acid returned	Hydrochloric acid not returned	Not followed	Average age	Basal metabolic rate	Per cent with achlorhydria	Per cent showing return of hydrochloric acid
2 years and over (average 6 years)	13	9	4	0	4	5	1	3	53	48	69	83
1 to 2 years 19 months duration	11	9	2	0	4	3	3	3	35	59	81	50
Under 1 year (4 months average duration)	26	16	7	3	11	11	3	2	36	50	62	78.5

TABLE IV

*Height of metabolism*

Basal metabolic rate	Number of cases	Achlorhydria	Hypochlorhydria	Normal	Hydrochloric acid returned	Hydrochloric acid not returned	Not followed	Per cent without hydrochloric acid	Per cent showing return of hydrochloric acid	
<i>per cent</i>										
Under + 50	7	5	2	0	1	2	2	71	33.3	Adenomas with hyperfunction
Over + 50	8	5	3	0	2	1	2	62.5	66.6	
Under + 50	17	12	3	2	9	1	2	70	90	Exophthalmic goiters
Over + 50	18	12	5	1	7	3	2	67	70	
Total under + 50	24	17	5	2	10	3	4	70	77	Adenomas with hyperfunction and exophthalmic goiters
Total over + 50	26	17	8	1	9	4	4	65	69	

twenty-six patients with metabolic rate of plus fifty or higher before iodine medication, sixty-five had no hydrochloric acid, while seventy per cent of twenty-four patients with metabolism of less than plus fifty showed achlorhydria. The number of cases in each group is too small to permit of attributing much significance to the difference in percentage.

Of the fifty patients with hyperthyroidism, nineteen or thirty-eight per cent gave a history of gastric symptoms of a severe character. The most prominent of these were anorexia, vomiting after meals, periods of diarrhea, and abdominal pain. Of these nineteen patients, fourteen had achlorhydria.

## II Gastric secretion in diseases of thyroid gland after treatment

The study of gastric secretion was continued during preoperative therapy and for at least six months after operation in forty-two of the

fifty patients with hyperthyroidism. In all the cases that have been followed, at least one subtotal thyroidectomy has been done, and each patient received preoperative iodine medication.

The gastric secretion was unchanged in two of the three patients with normal acidity before operation. The third had a persistence of hyperthyroidism and developed hypoacidity. The thirteen patients with hypoacidity before operation showed an increase in hydrochloric acid, total acidity and volume of secretion to normal after operation. Of the thirty-four patients with achlorhydria twenty six were followed, twenty with exophthalmic goiters and six with hyperfunctioning adenomas. In eighty per cent of the former and in fifty per cent of the latter hydrochloric acid reappeared in the gastric secretion. Over a period of time we have been able to demonstrate a gradual increase in the hydrochloric acid, total acidity, and volume of secretion. In other words, seventy-three per cent of twenty-six patients in whom achlorhydria had previously been demonstrated showed a return of gastric secretion to a normal level following the therapeutic reduction of thyroid over-activity.

In thirty two per cent of the cases, hydrochloric acid returned at the end of the preoperative iodine therapy. In sixteen per cent it was present ten days after operation. In the remaining fifty two per cent it reappeared in from one to two months postoperative. With few exceptions the hydrochloric acid reappeared first, only after histamine stimulation, then after the alcohol meal, and finally in all specimens.

Of the seven cases in which achlorhydria has persisted after operation, one has a basal metabolism of plus fifty-two, while another has signs of myxedema. Excluding these two whose persisting anacidity may be associated with abnormal thyroid function, the percentage return of hydrochloric acid for the group is seventy-nine per cent.

The observation that the achlorhydria of hyperthyroidism is transient seemed of such significance that these cases were studied carefully in an attempt to correlate the reappearance of hydrochloric acid with various factors. The results have demonstrated that the secretory function of the stomach is resumed when the thyroid hyperfunction ceases, as indicated by a decrease in the pulse and metabolic rates to a normal level. There is no indication of any definite correlation between the return of hydrochloric acid and the total iodine dosage, age, duration of disease or elevation of metabolism. (See Tables III and IV.)

On the basis of these four factors there is no significant difference between those patients in whom gastric acidity returned to normal and those with a persisting achlorhydria. Excluding the eight patients not followed and the two in whom the persisting anacidity may still be associated with a continuation of thyroid disease, there is an incidence of achlorhydria for the group of ten per cent which is not higher than the incidence for normal individuals in this age group (17, 18).

## DISCUSSION

The results of this study clearly indicate that the gastric acidity in hyperthyroidism is markedly diminished. There is a high incidence of achlorhydria and hypoacidity irrespective of whether the hyperthyroidism is associated with exophthalmic goiter or with adenomatous goiter with hyperfunction. This latter finding is of particular value as previous investigators have reported that achlorhydria is either rare (5) or does not occur in adenomatous goiter with hyperfunction (8).

Achlorhydria was demonstrated in sixty-eight per cent of fifty cases, an incidence five times that in normal individuals of the same age (17). This is a definitely higher figure than most investigators have reported (5, 7, 8, 10) and is more nearly comparable to that found by Herzfeld (6) and Moore (11).

Contrary to previous reports (8, 10, 13), our results indicate no relationship between the incidence of achlorhydria and the duration of the disease, height of the metabolism or age of the patient.

The most significant finding in this study has been the return of gastric secretion in the achlorhydrias of patients with hyperthyroidism after treatment. The reappearance of hydrochloric acid and the increase in gastric acidity and volume of secretion has not been demonstrated previously so far as we have been able to learn. Heimann (12) states that the secretory anomalies in hyperthyroid disease do not improve, but the evidence in support of this conclusion does not appear in his report. Our observation makes untenable several previously reported conclusions (2, 12, 13) based on the assumption that the acid secreting impairment was permanent.

A high incidence of achlorhydria in hyperthyroidism is to be expected in the light of recent experimental work on the nervous control of the gastric glands (19, 20, 21, 22). Considerable evidence has accumulated which would indicate that this control may be the resultant of two antagonistic forces, the excitatory secretory in the parasympathetic nerves and the inhibitory secretory in the sympathetic. Whether the excessive stimulation of the secretory inhibitory nerves to the gastric glands by the thyroid hormones can, in itself, explain this pronounced disturbance in the gastric secretion, or whether other extra-thyroidal factors play a part, we are unable to say. It seems reasonable, however, to suppose that an autonomic nervous imbalance in thyroid hyperfunction may manifest itself locally in the stomach as well as generally throughout the body.

The return of gastric acidity after treatment is suggestive evidence that the thyroid overactivity plays a major rôle in the depression of gastric acidity. It follows that the gastric glands should resume their normal function upon the removal of thyroid hyperfunction if no structural damage has occurred in the mucosa of the stomach. That no permanent damage resulted must be inferred, at least in seventy-three per cent of the

cases in this study Heimann (12) concluded that the achlorhydria resulted from a direct toxic action of the thyroid hormone on the gastric glands. If this were true, the injury must of necessity be a temporary one, from which the glands easily recover.

If a predisposing constitutional element were involved as an extra-thyroidal factor, the achlorhydria should persist. As already observed, however, the reappearance of HCl in patients with hyperthyroidism, who were known to have had an achlorhydria, has been as definite and clear cut as the increased tendency to achlorhydria.

A gastric analysis may be of some diagnostic value in differentiating between a mild or disguised hyperthyroidism and other diseases, particularly the neuroses.

#### CONCLUSIONS

1 The reported studies demonstrate that there is a high incidence of achlorhydria in hyperthyroidism.

2 Of fifty patients with hyperthyroidism, sixty-eight per cent had achlorhydria after histamine stimulation.

3 Achlorhydria was demonstrated in sixty nine per cent of thirty five patients with exophthalmic goiter, and in sixty seven per cent of patients with hyperfunctioning adenomas.

4 Control studies on nine patients having adenomatous goiters with normal function of the thyroid, and on one patient with acute thyroiditis showed normal gastric acidity.

5 The gastric secretion has been studied for six months after operation in forty two of the fifty cases of hyperthyroidism.

6 In seventy-three per cent of these the hydrochloric acid has returned in the gastric secretion and the total acidity has increased. The thirteen patients with hypoacidity before operation had a normal secretion after the thyroid overactivity ceased.

7 The results of this study support the belief that thyroid overactivity may play the major rôle in producing the diminished secretion of acid in hyperthyroidism.

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# THYROID HYPERPLASIA PRODUCED IN CHICKENS BY ULTRAVIOLET LIGHT DEFICIENCY

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In the course of an experiment designed to produce enlargement of the parathyroid glands of chickens by excluding ultraviolet light, it was noted that the birds were developing thyroid instead of parathyroid hyperplasia. As little reference could be found to the influence of light upon the thyroid gland, it was decided to study further this interesting observation. A portion of this work has already been briefly reported (1).

Sorour (2) found that the thyroids of rats kept in darkness resembled those of patients with Basedow's disease while the glands of rats kept in light were normal. The animals were upon a deficient diet and developed rickets and parathyroid hypertrophy.

Clausen (3) subjected rats to selective radiation with infra red, and found, in a limited number of animals that were examined, "marked enlargement and hypertrophy of the thyroid gland. Irradiation with ultraviolet, in corresponding litter mates, was found to prevent this hypertrophy whether or not the rat was exposed to infra-red radiation." These rats were also rachitic.

Bergfeld (4, 5) in a series of experiments on rats noted that the thyroid glands of the animals kept in darkness showed marked hyperplasia. This did not occur in nearly so marked a degree in animals exposed to daylight. Further analysis showed that the ultraviolet rays with a wave length below 310 millimicrons were the effective factor in preventing this hyperplasia. Metabolic studies showed no correlation between the histological picture and oxygen consumption. Unilateral cervical sympathectomy did not affect the development of hyperplasia. A further finding of interest was that rats kept in darkness failed to develop hyperplasia of the thyroids if injected with a skin extract prepared from other rats that had received ultraviolet light. Protection was not afforded by a similar skin extract from animals that did not receive ultraviolet. It is not clear from Bergfeld's reports whether or not his rats had rickets.

Rosenkranz (6) in a histological study of the thyroids of cattle confined to stables and rabbits in hutches found a hyperplastic reaction that was not present in grazing cattle or rabbits exposed to the sunlight.

Keeping young rats in darkness or exposing them to sunlight caused no change in the iodine content of the thyroids or of the skin, according to Van Hellebrandt (7).

Hess and Smith (8) showed that exposure of rats to excessive ultraviolet radiation or the administration of large amounts of viosterol had no effect upon the endocrine glands including the thyroids.

Sheard and Higgins (9, 10) found that chickens receiving no ultraviolet light showed a retardation of growth and hyperplasia of the parathyroid glands. Both effects were more or less prevented by the addition of 2 per cent cod liver oil by weight to the diet. Apparently thyroid hyperplasia did not occur as there is no mention of it in these papers.

In summary, evidence is accumulating suggesting that the absence of ultraviolet light causes thyroid hyperplasia.

#### PRELIMINARY OBSERVATIONS

In January, 1929, a group of six-day old Barred Rock chicks was separated into two equal lots, each of which was placed in a pen in the same room. The birds in one pen received diffuse daylight through ordinary window glass and were used as controls. The second pen was covered over with number 48 Pittsburgh amber glass. This glass according to Sheard and Higgins (9, 10) transmits about 30 per cent of the visible rays and 50 per cent of the infra-red, while none of the waves of a length less than 360 millimicrons pass through. Spectrographic analysis of the particular sample of glass used by us was obtained through the kindness of Professor Hans T. Clarke. It was found that all rays below 344 millimicrons were excluded by the glass. The diet for both groups of chickens was the same and consisted of a mash containing corn and bone meal, wheat middlings, limestone grit, and salt, to which was added cracked corn and wheat. Cod liver oil was mixed with the grain for the first 15 days.

At frequent intervals after the birds had reached an age of 55 days, a chicken from each lot was selected, weighed, and then killed by the administration of ether. Before death had occurred a specimen of blood was obtained by cardiac puncture. At autopsy the following points were noted: general condition, presence of rachitic deformities, consistency of bones, gross appearance of parathyroid and thyroid glands. These glands were then removed for microscopic study. In a few instances a femur was taken for determination of its calcium and phosphorus content.

A total of 37 chickens was examined. The experiment ended in June, 1929.

*General condition.* The chickens in both groups were usually well, vigorous, and had good plumage. There was a marked variation in size within each group. The bones of the birds raised under amber glass seemed softer than those of the controls, but except for a twisted sternum

in a few instances there was no other rachitic deformity. One chicken developed leg weakness.

**Weights.** Figure 1 shows that the chickens raised under amber glass were smaller than the controls particularly in the later part of the period

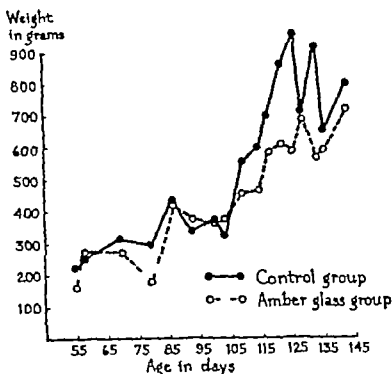


FIG. 1. WEIGHTS OF TWO GROUPS OF CHICKENS AT DIFFERENT AGES IN PRELIMINARY OBSERVATIONS

of observation. The average weights for the entire groups were as follows:

- 19 chickens raised under amber glass—average weight, 426 grams
- 18 control chickens —average weight, 535 grams

**Serum calcium and phosphorus.** The calcium was determined by the method of Clark and Collip (11) and the phosphorus by the method of Fiske and Subbarow (12). A wide variation was noted in both groups as follows:

- A 17 chickens raised under amber glass
  - Serum calcium —range 5.3 – 12.0 mgm per 100 cc.
  - average 9.6 mgm
  - Serum phosphorus—range 1.5 – 8.5 mgm per 100 cc.
  - average 4.7 mgm
- B 18 control chickens
  - Serum calcium —range 7.0 – 12.6 mgm per 100 cc.
  - average 10.9 mgm
  - Serum phosphorus—range 4.7 – 8.3 mgm per 100 cc.
  - average 6.6 mgm.

Ackerson, Blish and Mussehl reported (13) that the average serum calcium in 68 normal chicks was 10.6 mgm and the phosphorus 4.60 mgm,



and 65 each the chicks the calcium averaged 7.10 mgm. The phosphorus in both groups was within normal limits, but the serum calcium of the chickens under amber glass was below the normal average.

*Calcium and phosphorus in bone*—From each of eight chickens a femur was removed, dried to constant weight, and analyzed for calcium and phosphorus by the same methods used for the serum. The results (Table I) show that the bones of chickens in the amber glass group had a slightly lowered calcium content and a noticeable reduction in phosphorus.

TABLE I  
*Ca and P content of bone and serum*

	Chick number	Age of chicken	Weight of dried femur	Ca per gram of bone	Phosphorus per gram of bone	Serum calcium	Serum phosphorus
		days	grams	mgm	mgm	mgm per 100 cc	mgm per 100 cc
Control group	22	114	1.987	165	87	12.6	6.3
	24	117	2.938	165	76	11.4	6.8
	26	121	2.886	172	72	11.4	8.0
	30	128	2.431	164	87	10.7	7.0
			<i>Average</i>	167	81	11.5	7.0
Amber glass group	23	114	1.262	159	84	9.7	6.8
	25*	117	2.130	152	60	9.4	2.7
	27	121	2.149	162	65	9.5	5.1
	31	128	2.282	167	75	7.7	6.0
			<i>Average</i>	160	71	9.1	5.7

\* Despite the low Ca and P in both bone and serum this bird was well developed, healthy and vigorous. The parathyroids were small, the thyroids large and congested.

*Thyroid and parathyroid glands*—Enlargement of the parathyroids described as slight to moderate, occurred in 6 of the 18 chickens raised under amber glass. In the remainder of the group and in the controls no important variation was noted.

Beginning at the age of 85 days and occurring consistently thereafter a bilateral enlargement of the thyroids, often of marked degree, was found in the chickens of the amber glass group. In addition to being larger than the glands of the control birds, these thyroids were deep purple in color contrasted to yellowish-pink of the normals. Histologically the enlarged thyroids presented the picture of active hyperplasia.

These changes were unexpected and suggested the work described as the first experiment.

*Summary of preliminary observations*—A group of Barred Rock chicks, raised under amber glass that excluded all rays of wave-lengths less than 344 millimicrons, showed the following differences when compared with a control group that received diffuse daylight through ordinary window

glass (1) retardation of growth, (2) lowered serum calcium and phosphorus, (3) softer bones containing less calcium and phosphorus, (4) inconstant parathyroid enlargement, (5) marked enlargement of the thyroid glands

#### FIRST EXPERIMENT THE PRODUCTION OF THYROID HYPERPLASIA BY ULTRAVIOLET LIGHT DEFICIENCY

In order to avoid complicating factors and to render the results as clear-cut as possible certain modifications of the experimental conditions were introduced. Barred Rock chicks were again used. The pens were the same and were indoors as before. The changes were as follows:

- 1 Because the preliminary mortality among both groups of chickens was apt to be high, it was decided to buy four-week old chicks instead of those newly hatched. Upon receipt the birds were kept together for a week or ten days. During this period cod liver oil was added to the diet. At the age of 5 to 6 weeks, the chicks were divided into two lots, one of which was placed in the amber glass pen.

- 2 The chickens used as controls received ultraviolet radiation for five minutes three times a week by means of a mercury arc lamp at four feet in addition to diffuse daylight through ordinary window glass.

- 3 The mash used in the preliminary work was replaced by a commercial growing mash containing oatmeal, hominy feed, yellow hominy feed, barley meal, wheat bran, wheat middlings, fish meal, meat scraps, alfalfa meal, sodium chloride, ground limestone, molasses, and cod liver meal (1 per cent by weight). Lettuce or cabbage was also given to both lots two or three times a week.

A total of 58 chickens was used in this experiment. The birds were from three different hatches occurring in February, April and September, 1930. As no variation in response was apparent the results obtained from the three lots have been consolidated for convenience.

*General condition* There was no difference in the general condition of the control chickens and those raised under amber glass. The birds were vigorous, healthy, and well plumaged. There was no evidence of rickets or leg weakness and the bones of both lots were firm.

*Weight* Contrary to the results obtained in the preliminary work, there was no difference between the weights of the controls and of the chickens that received no ultraviolet light (Figure 2). The average body weight of 28 control birds of various ages was 632 grams, while that of 30 chickens raised under amber glass was 630 grams. The close correlation in growth curves is believed to be a result of the constant daily intake of small amounts of cod liver oil.

*Serum calcium and phosphorus* Analyses of the calcium and phosphorus content of the serum were made on 6 chickens from each group



from 7-107 mgm and averaged 46 mgm More complete details for the entire series of 58 chickens are shown in Table II which gives the age, body weight in grams, thyroid weights in milligrams, and milligrams of

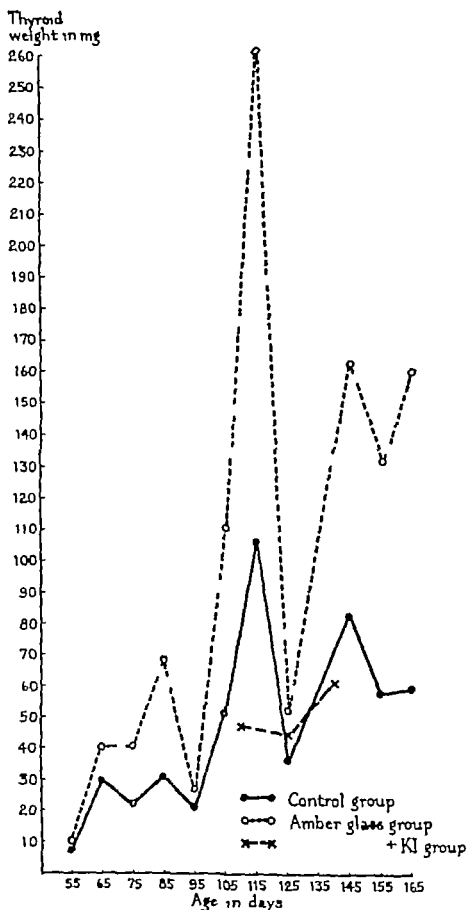


FIG 3 VARIATIONS IN THYROID WEIGHTS

thyroid per kilogram of body weight This last item is of particular interest because of the close correspondence of body weights in the two groups The variation in the thyroid body weight ratio as expressed in



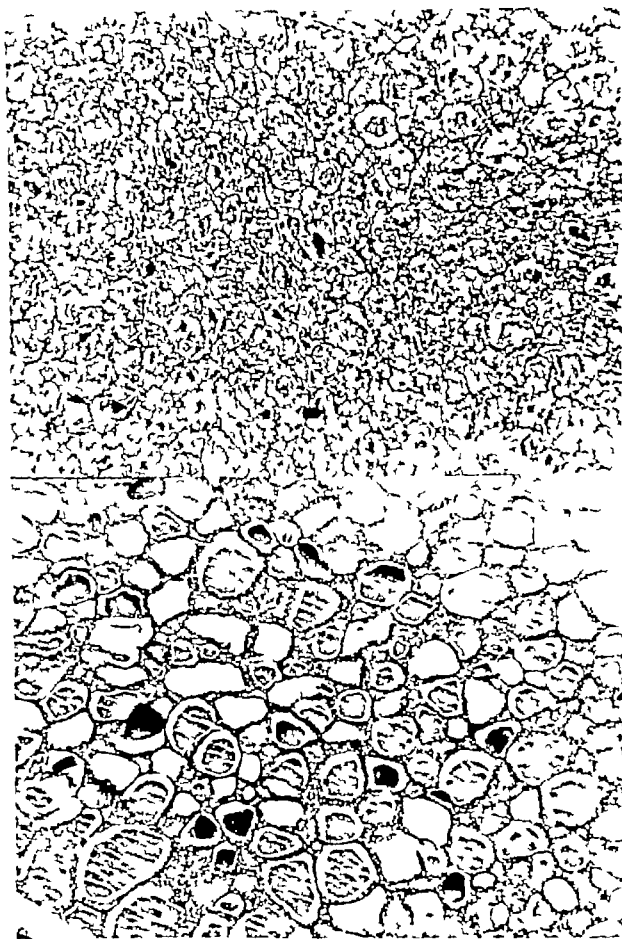


FIG 4

At the bottom thyroid from a control chicken that received ultraviolet light. The follicles are large and well filled with colloid. The epithelial cells are cuboidal. The upper section is from a chicken of the same age and weight that was deprived of ultraviolet light. Hyperplasia of the epithelial cells is evident. The follicles are more numerous and smaller. The amount of colloid is decreased. The oxygen consumption of both birds was the same (Magnification  $\times 200$ ).



In addition to gross enlargement, there was a marked difference in color. Whereas the normal gland was a pale, yellowish pink, the enlarged glands were usually deep, purplish red and gave the impression of increased vascularity.

The histological differences are well shown in Figures 4 and 5. In the thyroids of the control group the epithelial cells were low cuboidal, the follicles were large, and colloid was abundant and readily stained. The enlarged glands of the ultraviolet deficient chickens showed very marked hyperplasia of the epithelial cells which were more cylindrical in form, smaller and more abundant follicles, and a great diminution in the amount of colloid. The colloid that was present stained poorly. There was no lymphocytic infiltration.

*Oxygen consumption.* Through the kindness of Dr Dickinson W Richards, Jr, who supervised this part of the experiment, it was possible to determine the oxygen consumption of four chickens from each group (Table III). In spite of the fact that the ultraviolet deficient chickens

TABLE III  
*Oxygen consumption compared with size of thyroids*

	Chicken number	Number of metabolism determinations	Average O <sub>2</sub> consumption per gram of chicken per minute	Thyroids in mgm. per kgm. body weight
Control group	64	3	1.07	55
	65	7	1.07	77
	66	3	0.96	49
	67	4	0.82	58
		<i>Average</i>	0.98	60
Ultraviolet deficient group	64A	4	0.91	74
	65A	3	0.93	145
	66A	6	1.02	111
	67A	3	0.87	179
		<i>Average</i>	0.93	127

had twice as much thyroid tissue per kilogram of body weight as the controls, the oxygen consumption displayed no significant variation, showing that the hyperplasia was a compensatory process and that the enlarged gland was not an overacting one.

*Iodine content of thyroids.* Iodine determinations were made upon three glands by Dr Alexander B Gutman to whom we are indebted for the figures shown in Table IV. The results suggest that the iodine content of the hyperplastic gland was low.

*Summary.* (1) A group of chickens raised under amber glass that excluded all the ultraviolet rays below 344 millimicrons was compared





TABLE V

*Body and thyroid weights of ultraviolet deficient chickens receiving excessive amounts of iodine*

Age	Body weight	Thyroid weights	Mgm. thyroid per kgm. body weight
<i>days</i>	<i>grams</i>	<i>mgm.</i>	<i>mgm.</i>
111	876	47	54
111	773	60	78
111	509	23	45
111	674	60	89
124	1290	68	53
124	685	30	44
124	890	41	46
124	781	41	53
140	938	83	89
140	910	49	54
140	943	50	53
Average	843	56	60

Table VI which serves as a partial resume of the work. The average body weight of the controls and the ultraviolet deficient chickens is strikingly similar. The ultraviolet deficient group fed KI cannot be included in this comparison because there were no chickens less than 110 days old in this group, which also accounts for the slightly increased thyroid weights of

TABLE VI

*Summary of body and thyroid weights for the three groups of chickens*

Group	Number of chickens used	Average body weight	Average thyroid weights	Average mgm. of thyroid per kgm. body weight
		<i>grams</i>	<i>mgm.</i>	<i>mgm.</i>
Controls	28	632	46	75
Ultraviolet deficient	30	630	102	179
Ultraviolet deficient fed KI	11	(843)	56	60

these birds. The thyroid glands of the chickens raised under amber glass without the addition of iodine to the diet are more than twice the weight of the control glands. This difference again appears when the results are expressed as milligrams of thyroid per kilogram of body weight. In this last comparison the iodine fed birds closely resemble the controls.

## CONCLUSIONS

Enlargement of the thyroid glands of growing, non rachitic chickens may be produced by the exclusion of ultraviolet light. Histologically the glands are hyperplastic, and poor in colloid. The thyroid enlargement is not accompanied by an increase in the bird's oxygen consumption. The hyperplasia may be prevented by the administration of potassium iodide.



## LACTIC ACID IN THE BLOOD OF RESTING MAN

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The conception that, under resting conditions, blood lactic acid varies directly with pH is now current in the literature. According to this view, in states of alkalosis, lactic acid increases in the blood while during acidosis the opposite effect is produced. The origin of this conception is chiefly attributable to the work of Macleod (1), Anrep and Cannan (2), Long (3), and Eggleton and Evans (4). A further commonly accepted view concerning lactic acid in the blood of resting man is that this substance originates chiefly from muscle metabolism. The gradual accumulation of data in our hands from various sources during the past few years with respect to lactic acid has led to the formulation of the following inquiry: To what extent are the generally accepted mechanisms mentioned above concerning lactic acid operable in man? An investigation of these questions is desirable because much of the existing information has accrued from experimental work on cats and dogs. The present data have been obtained in a study of normal man unless otherwise stated. The methods used for preventing *in vitro* changes in the blood and for its analysis have been described before (5). Following the procedure we have adopted, the amount of lactic acid found in the blood of resting man has a range of 6 to 14 mgm per 100 cc, the average value being about 10 mgm. Alkalosis has been produced by ingestion of sodium bicarbonate or by voluntary over ventilation. Acidosis has been produced by ammonium chloride administration. In addition, observations have been made in anoxemia, in moderate work, and on a dog during alkalosis.

### ALKALOSIS

After a rest of 30 minutes in a reclining position, venous blood was secured from four normal men, and alveolar air samples were taken. The subjects then ingested 20 gram doses of sodium bicarbonate at 9 A M, at 11 A M and at 2 P M. During the day the alkaline reserve<sup>1</sup> of venous blood was determined from time to time with results as indicated in Figure 1. On the first and last samples of blood, analyses were per-

<sup>1</sup> Alkaline reserve is defined as the carbon dioxide content of oxygenated whole blood when the partial pressure of carbon dioxide is 40 mm Hg.



TABLE I  
*Sodium bicarbonate experiments*

Date	Subject	Time	Alveolar pCO <sub>2</sub>	Alkaline reserve <sup>a</sup>	Lactic acid	pH <sub>t</sub> <sup>†</sup>
			mm. Hg	volumes per cent	mgm. per 100 cc.	
1928						
June 22	H D Normal		39.6	45.9	8.1	7.39
June 6	H D After alkali		42.6	57.2	7.2	7.46
September 27	H D After alkali		38.3	46.7	11.7	7.41
October 1	H D After alkali		40.3	56.1	12.6	7.47
November 13	I G Nephrotic			67.2	8.5	7.53
1931						
September 30	D B D Normal	9 A.M.	38.0	50.45	13.6	7.44
September 30	D B D After alkali	5 P.M.	46.7	64.3	10.5	7.50
October 2	A. V B Normal	9 A.M.	38.0	47.65	8.2	7.43
October 2	A. V B After alkali	4 P.M.	45.1	61.0	7.6	7.49
October 2	S. A. O Normal	10 A.M.	39.0	47.8	9.2	7.42
October 2	S. A. O After alkali	4 P.M.	45.0	63.45	10.3	7.52
October 2	A. J D Normal	9 A.M.	38.0	47.8	11.3	7.43
October 2	A. J D After alkali	4 P.M.	43.4	64.2	14.6	7.51

<sup>a</sup> See footnote 1 of text<sup>†</sup> Calculated in this and following tables from the known properties of blood and to correspond to the observed pCO<sub>2</sub> in alveolar air

reserve force to combat alkalosis. But even in the absence of alkalosis such experimental procedures are frequently followed by large increases in blood lactic acid. There is no substantial evidence suggesting that a similar mechanism is operative in man under any commonly found set of circumstances.<sup>2</sup>

## ACIDOSIS

In a period of 48 hours two subjects ingested 35 grams of ammonium chloride. After a rest period of 30 minutes samples of alveolar air and blood were taken. The data obtained are given in Table II, together with similar data from the subject H. D. taken from Dennig's paper mentioned above. The values found for lactic acid again fall in the normal range even though the alkaline reserve may be reduced by more than 50 per cent with the pH<sub>t</sub> reduced to 7.21. The data are few but the experimental conditions are simple, the technique for the various determinations is thoroughly standardized and the results leave little question that additional observations would give similar results. It is perhaps worth

<sup>2</sup> In the paper of Himwich, Koskoff and Nahum (7) in which high lactic acid values were found in anesthetized and decerebrated animals the authors make the following statement: "The experiments on the resting unanesthetized dog and those on the human beings have not been tabulated since they did not indicate significant changes in the lactic acid and glucose content of the blood."

carbon dioxide is probably of the order found by Long. Since, under these conditions, we could demonstrate little if any change in blood lactic acid, the question was not pursued further. Our data do not lend support to Long's hypothesis.

2 *Voluntary over-ventilation.* After the usual initial period of rest on a bed the subject began voluntarily to increase his breathing. The expired air was collected in a 600-liter spirometer, an observer announced the approximate volume expired each minute and the subject attempted to maintain his volume of breathing at about 30 liters per minute. Near the end of the experiment, alveolar air samples were collected and blood drawn from both the radial artery and antecubital vein. Evidences of a state of tetany were present in every case but one when the blood was taken. The data are given in Table IV. They show that the arterial  $\text{CO}_2$  pressure ranges between 12 and 16 mm, the arterial  $\text{CO}_2$  content is below 35 volumes per cent, the pH<sub>a</sub> ranges from 7.60 to 7.73 and the lactic acid rises to two or three times the normal value. The duration of the experiments was too short to produce any appreciable change in alkaline reserve.

In these experiments the increase of lactic acid is associated with a high grade of alkalosis concerning which there can be no doubt, but it is to be noted that the experimental conditions are such as to interfere with the function of the greatest regulatory mechanism of the body, that of spontaneous breathing. Not only is the pH of the blood altered, but presumably many other variables are also grossly shifted. In the blood the primary shift occurs in the excessive elimination of carbon dioxide by the lungs and since the body is provided with protective devices of various sorts, it has been held that an increase in lactic acid during hyperventilation is a means of offsetting alkalosis. It seems clear that the increase in lactic acid is related to a complex physicochemical change which cannot be resolved with present knowledge and that therefore the explanation of the increase of lactic acid must remain uncertain. The results are quite different from those found when similar volumes of breathing per minute were induced by the simple expedient of permitting carbon dioxide to accumulate in the inspired air. In three out of five experiments in which lactic acid was determined in both arterial and venous blood there is evidence that the formation of lactic acid occurs in the muscles, but this bore no proportionate relation to the tetanic spasms and paresthesia noted by the various subjects. Such a suggestion was made by Peters, Bulger, Eisenman and Lee (8) to account for the increased organic acid found in the blood after hyperventilation.

#### ANOXEMIA

In addition to conditions influencing primarily the acid-base equilibrium, lack of oxygen is usually considered as the principal cause of an





increase in blood lactic acid. The highest values for lactic acid in blood have been those found during or after severe physical exercise, in which either the supply of oxygen is inadequate or the rate of formation of this acid exceeds the speed of its resynthesis or removal independent of the oxygen supply. Notwithstanding the fact that anoxemia is constantly mentioned as a cause of increased amounts of lactic acid in the blood, there is little evidence to show at what level of anoxemia this occurs in resting man. Koehler, Behneman, Benell, and Loevenhart (9) have shown that acidosis is the terminal condition in pigs, when these animals are subjected for a sufficient period to atmospheres of oxygen as low as 4 or 5 per cent. Macleod (10) found an increase in lactic acid in animals subjected to severe anoxemia and shock, but the operative conditions were probably of vital importance in his results. Meakins and Long (11), Jervell (12) and Groag and Schwarz (13) have reported increases in lactic acid in the blood of patients suffering from congestive heart failure more or less proportionate to the degree of circulatory failure. Weiss (14) has been unable to confirm the latter results. His data indicate that lactic acid seldom exceeds 20 mgm in patients suffering from congestive failure. Many other conditions such as anesthesia, hemorrhage, diseases of the liver, etc., are associated with abnormal amounts of lactic acid in the blood, all presumably related in one way or another to oxygen deficiency.

TABLE V  
*Anoxemia experiments*

Date	Subject	Inspired gas	Duration	HbO <sub>2</sub> content of arterial blood	Lactic acid	pH <sub>s</sub>
1931			minutes	per cent of capacity	mgm per 100 cc	
May 1	D B D	9 per cent O + N <sub>2</sub>	52	56.2	12.0	7.49
May 22	D B D	9 per cent O + 2.47 per cent CO + N <sub>2</sub>	67	69.8	9.7	7.42
May 27	A V B	9.1 per cent O <sub>2</sub> + N <sub>2</sub>	72	64.3	12.8	7.45
June 2	A V B	9.2 per cent O <sub>2</sub> + 2.5 per cent CO + N <sub>2</sub>	58	75.0	10.4	7.41
June 10	H T E	9.2 per cent O + 2.7 per cent CO <sub>2</sub> + N <sub>2</sub>	43	76.9	12.0	7.45
June 16	H T E	9.2 per cent O + N <sub>2</sub>	68	51.1	15.5	7.45

In connection with another research not yet published we have obtained the data shown in Table V. The subjects, while sitting in a chair, breathed in one case an atmosphere of 9 per cent oxygen + 91 per cent nitrogen and in another case a gas mixture consisting of 9 per cent oxygen + 2.5 per cent carbon dioxide + 88.5 per cent nitrogen. Nine per cent oxygen at sea level is equivalent to the partial pressure of this gas at an altitude of 22,000 feet. The degree of anoxemia reached in these experiments, as shown by the per cent saturation of arterial blood with oxygen,

equals or exceeds that found in most cases of lobar pneumonia or of circulatory failure. The lactic acid values found are on the average about 2 mgm higher than the average normal value. Such results suggest that anoxemia *per se* in man must be of a very high order of magnitude if it is to cause a substantial increase in lactic acid. Lactic acid formation is shown to be little affected when the partial pressure of oxygen in arterial blood drops to half normal. Of course it is possible that in some pathological conditions there may be not only decreased saturation of arterial blood but also a slower rate of movement of blood through the capillary bed. When this occurs the mean pressure of oxygen will be further reduced.

#### MODERATE PHYSICAL EXERCISE

Although the response of the body during physical exercise is not of primary interest in this paper, the data of experiments tabulated in Table VI are included to demonstrate the stability of lactic acid concentration

TABLE VI  
*Exercise experiments of 30 minutes' duration*

Date	Subject	Rate	Oxygen used	Lactic acid in venous blood	
				Resting	After work
1931		miles per hour	liters per minute	mgm. per 100 cc.	mgm. per 100 cc.
October 21	W E C *	3.5	0.94	9.0	9.8
October 26	W E C	4.6	1.49	16.0	11.7
November 2	W E C	7.0	2.30	13.3	13.3
November 4	W E C	5.8	1.84	15.2	12.1
November 9	W E C	8.6	2.70	12.1	25.4
October 23	A J D	2.3	0.99	11.3	12.1
October 26	A J D	3.5	1.09	9.0	9.8
November 2	A J D	4.6	1.69	11.3	15.6
November 24	A V B	3.5	1.18	13.7	9.8
November 24	Miss D C	3.5	1.01	9.2	9.9
November 24	S A O	3.5	1.27	9.0	8.6

\* Trained runner

even during exercise requiring a metabolic rate several times that of the resting state. The subjects walked or ran for 30 minutes on an electrically driven treadmill at the speeds indicated in the table. The only subject in physical training was W E C. The results are significant, especially when account is taken of the conception that the lactic acid present in the blood during rest has its origin in the muscles. These experiments appear by contrast to exclude such possible sources for lactic acid during the resting state as the activity of the accessory muscles of respiration, sporadic movements of the body commonly observed in subjects lying at rest, tetanic spasms, etc. An untrained subject may by exercise in

crease his metabolic rate four or five times without altering his blood lactic acid. The duration of the walking tests was adequate to permit an even distribution of lactic acid throughout the body and thus raise the level of lactic acid in the blood if an overflow had occurred from the exercised muscles. The evidence suggests that resynthesis or removal of lactic acid had kept pace with its production.

#### DISCUSSION

From the data given above it is clear that lactic acid concentration in resting man is, relatively speaking, stable, and is not greatly influenced by mechanisms formerly considered operative. The only circumstance found in this study in which the normal resting level of lactic acid is disturbed is that of voluntary over-breathing. This fact gains in significance because the experimental conditions involved were the only ones in which there was any voluntary interference with normal regulatory mechanisms of the body. Eggleton and Evans (4) stress the importance of avoiding over-ventilation in experiments in which variations in the lactic acid content of the blood are being studied. In man there is evidence on every hand that spontaneous breathing is capable of reacting to changes occurring in the internal environment in a manner to prevent shifts of state inimical to the organism until extreme conditions are met.

Early experiments of Macleod and Hoover (15) showed that lactic acid was increased in the blood when alkaline-dextrose solutions were given intravenously. No increase in lactic acid occurred when dextrose was injected in acid solution. The animals were anesthetized and had the vena cava and portal vein exposed. In view of these results Macleod and Knapp (16) suggested that lactic acid may act as an acid reserve useful in counteracting alkalosis. They found that intravenous injections of solutions of sodium carbonate and sodium bicarbonate in rabbits and dogs caused an increased output of lactic acid in the urine, and that this might occur without change in the pH of the blood. The same result was obtained when alkali was fed to two cats and three normal men. Jervell (12) found that ingestion of bicarbonate by seven patients was followed by a decrease in concentration of lactic acid in the blood of five of the subjects. In another series of observations made on the urine of other subjects he found increased excretion of lactic acid following bicarbonate ingestion.

In order to determine the extent of variations in lactic acid concentration that may be induced in a normal dog after bicarbonate administration the following experiment was done. A mongrel dog weighing 13 kilograms was given sodium bicarbonate in 10 per cent solution, by means of a stomach tube as indicated in Table VII. In all, 848 cc of bicarbonate solution were given in the course of three and one-half hours. During this time approximately 300 cc were vomited and two fluid

TABLE VII

*Response of a dog to alkali administration*  
*(NaHCO<sub>3</sub> given in 10 per cent solution by stomach tube Weight of dog 13 kgs)*

Time	Remarks	Rectal temperature	Observations on venous blood						
			HbO <sub>2</sub> capacity	CO <sub>2</sub> content	Alkaline reserve	pCO <sub>2</sub> of blood as drawn	pH <sub>a</sub> of blood as drawn	pH <sub>v</sub> of blood at pCO <sub>2</sub> 40 mm.	Lactic acid blood as drawn
		F	vol- umes per cent	vol- umes per cent	vol- umes per cent	mm. Hg			mm. per 100 cc.
12:00 M		101.8	21.5	43.8	39.2	50.2	7.25	7.31	17.4
12:05 P M	100 cc. alkali								
12:18 P M	125 cc. alkali								
12:20 P M	60 cc. vomitus								
12:23 P M		101.9	22.0	48.6	45.2	47.1	7.33	7.38	24.5
12:45 P M		102.5	22.5	53.9	50.4	47.6	7.38	7.44	20.5
1:01 P M	125 cc. alkali								
1:10 P M	50 cc. vomitus								
1:23 P M		102.2	22.8	63.7	56.9	54.0	7.40	7.50	22.5
1:37 P M	125 cc. alkali								
1:43 P M	25 cc. vomitus								
1:54 P M	Fluid stool								
2:10 P M	125 cc. alkali								
2:15 P M	25 cc. vomitus								
2:20 P M		101.9	26.1	68.2	58.4	57.0	7.42	7.52	33.6
2:58 P M	128 cc alkali								
3:02 P M	125 cc. vomitus								
3:14 P M		101.7	26.4	69.4	63.0	51.5	7.48	7.56	43.1
3:35 P M	120 cc. alkali								
3:48 P M	Fluid stool								
3:55 P M		100.8	28.2	69.8	57.8	61.0	7.40	7.53	47.4

stools were passed. For the last hour the animal appeared sick and was unwilling to stand. By the following morning he was apparently in normal condition. The initial resting value for lactic acid, 17.4 mgm., was somewhat high and showed comparatively little tendency to change after the animal had had 35 grams of bicarbonate, equivalent to three times the dose given to normal men as noted in Table I. The shift in pH<sub>v</sub> toward the alkaline side during this time was about 0.2. Subsequently, as more alkali was given, lactic acid concentration increased rapidly. There was little further change in pH<sub>v</sub> or in CO<sub>2</sub> content of the blood. The condition of the animal was no longer good. The data indicate that this dog could be subjected to a dose of alkali three times greater than that given to a normal man without exhibiting any great change in lactic acid concentration, notwithstanding a change in pH<sub>v</sub> of approximately 0.2.

Our data as a whole indicate that the lactate content of the blood is not readily altered during alkalosis, and we conclude that any mechanism

by means of which such an event might be brought about is of little significance in man, normally or pathologically.

Moderate acidosis produced by the use of ammonium chloride has no effect on blood lactic acid. In extreme states of acidosis following anoxemia, such as those produced in pigs by Koehler, et al (9) great increases in lactic acid may occur, but are of little significance in the present discussion since they represent but one measurable aspect of the dissolution of the whole body.

In anoxemia Macleod (10) found an increase in lactate concentration when the respiratory mixture breathed by his anesthetized animals contained 4 to 8 per cent of oxygen, the oxygen pressures being similar to those used by Koehler, et al. Macleod suggested that the excess of lactic acid may be formed to neutralize the excess base resulting from blowing off of carbon dioxide from the blood. We find difficulty in accepting such an hypothesis without qualification in view of our results under conditions of anoxemia representing the limit to which normal men can be subjected for a period of one hour. In these experiments no compensatory mechanism involving lactic acid is evident. The data suggest that anoxemia in various clinical conditions must reach a grave state to disturb lactic acid concentration in the body. In corroboration, Jervell found in short experiments on men, while breathing low oxygen mixtures, no increase in lactic acid until the oxygen breathed was reduced to 7.5 per cent. A further consideration of this question will be published later.

There appears to be little doubt that lactic acid, in the form of a salt, is a normal constituent of blood. Some question may be raised as to the exact amount of it in a given blood sample, since the method used for its measurement may include sulphite-binding products which result from the oxidation of substances other than lactic acid. Long (3) roughly estimated the amount of lactic acid in blood by the thiophene test and compared the values obtained with those given by Clausen's method (16). He concluded that one-half to three-quarters of the yield given by the latter method was due to lactic acid itself. However, the modification of the Clausen method, since introduced by Friedemann, Cotonio and Shaffer (17), has reduced the number of interfering substances, and according to these authors has made the method more nearly specific for lactic acid. As a result, the value now obtained for lactic acid in blood during rest is approximately one-half that given by the Clausen technique. Until further work is done we may assume that the concentration found for lactic acid by the present method represents this substance.

Owing to the importance of the rôle of lactic acid in muscular contraction, as indicated in the work of Meyerhof and Hill (now modified (18)), activity of muscle, whether under working or resting conditions, is com-

monly regarded as the source of lactic acid in the blood. Severe physical exercise in untrained men is accompanied by a large increase in lactic acid concentration, but in the case of trained individuals the same grade of exercise may produce no appreciable change in lactic acid. Moderate exercise, as our experiments and those of Owles (19) show, may be carried on with little or no alteration in blood lactic acid. Whether or not lactic acid concentration in blood increases under given conditions of exercise depends not only upon an adequate oxygen supply but presumably also upon the speed of its production.

If moderate muscular exercise in untrained men can be executed without appreciable change in the level of blood lactic acid, it appears improbable that the lactic acid formed during resting states has its origin only in muscle metabolism. The rôle now assigned to lactic acid in muscle activity (18) has no bearing on the subject. Taking into account the relative stability of lactic acid concentration during rest, it seems better not to attempt a specific explanation but to go no further than to regard this phenomenon as a part of the general process of metabolism as a whole. We suggest, for example, that it may be a split product in the oxidation of carbohydrate, mobilized for the maintenance of general body needs. Much work with reference to glucose oxidation must be done before the question can be settled. Of the lactic acid present in the blood of a resting subject a small portion may come from muscle activity, the rest presumably from the activity of the central nervous system, various glandular activities, etc. It seems logical to suppose that the ability to reconvert lactic acid to its precursor may vary greatly at the seat of its formation, just as the rate of utilization of oxygen may vary from organ to organ in the body. The problem remains for study along lines differing from those suggested in the past. An excellent review of the literature concerning lactic acid has been published recently by Himwich (20).

#### SUMMARY

It has been shown that lactic acid concentration in the blood of a resting man is not related simply to shifts in hydrogen ion concentration. Anoxemia is not accompanied by increasing amounts of lactic acid until extreme conditions are met. Evidence is given indicating that of the total lactic acid found in the blood under resting conditions, only a part may originate from muscle activity. No definite evidence is at hand to indicate the source of the remainder, but it is suggested that it may be a split product of carbohydrate mobilized for the maintenance of the general metabolism of the body.

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## A STUDY OF THE CIRCULATION IN OBESITY<sup>1</sup>

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The status of the circulatory system in obesity has been the subject of considerable interest, study, and conjecture for many years. Laennec (1) mentioned the "fatty heart" as an anatomic condition, while Stokes (2) thought he could recognize it clinically. In more recent years chief interest has centered on the pathologic physiology of the condition and the clinical conception of the fatty heart as such has, for all practical purposes, passed into discard. It is recognized that symptoms which may be taken as evidence of cardiac insufficiency may manifest themselves in obesity where the pathological changes found in the circulatory system seem hardly commensurate with these symptoms. Many have considered the primary disturbance in these cases to be in the heart, and Hirsch (3), who showed that the muscle mass of the heart is relatively small for the weight of the obese patient, looked upon this disproportion as an important factor in the relative inefficiency of the heart in obesity. Romberg (4) also holds this view. The mechanical disturbances produced by the thick epicardial fat layer as well as by the height of the diaphragm with subsequent transverse displacement of the heart are also considered as significant hindrances to satisfactory cardiac function. At any rate, the condition of the circulatory system is a subject of prime importance in obesity and in most cases the prognosis of the disease is chiefly dependent upon it. Following the monograph of Eppinger, Kisch, and Schwarz (5), in which such an intimate inter-relationship was suggested between circulatory and metabolic disturbances, an attempt was made to find in the metabolic abnormalities of obesity factors which might explain its circulatory maladjustments. As Groscurth (6) notes, circulatory diseases are responsible sooner or later for disturbances in metabolism, and vice versa.

In the present study an attempt was made to analyze through a many sided study of the circulatory system the factors responsible for the picture of so-called relative cardiac insufficiency in obesity. Are the symptoms due purely to mechanical disturbances, are they due to some intrinsic weakness of the heart, or are they due to metabolic abnormalities?

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It seemed that a satisfactory way to study the circulation in obese patients would be to observe the effect of measured exercise under carefully defined conditions on various phases of the circulation, and to compare these results with the results obtained under similar conditions in a group of normal people.

Three obese patients were compared with three normal persons. The results in the three cases of obesity were so consistent that it was thought much more would not be gained by studying a larger group of similar cases. A fourth normal person was studied who was of such size that his normal weight approximated that of one of the obese patients. The obese patients exhibited beyond simple obesity no abnormalities which could in any way be associated with the circulatory system.

The cases were studied in the sitting and standing resting positions, during and after exercise. The exercise consisted of walking on a treadmill and was of such a character as to be most typical of the exercise which a fat person would be likely to perform in the routine of his daily life. It was also selected as a suitable form of exercise because so many muscles of the body were put to use. The exercise was in no way severe, so that the problem of exhaustion was excluded.

We arbitrarily selected for study the following phases connected directly or indirectly with the circulatory system of the blood: Vital capacity, respiratory minute volume, respiratory rate, oxygen consumption, respiratory quotient, arteriovenous oxygen difference, cardiac output, pulse rate, stroke volume, blood pressure, lactic acid and other blood changes.

#### METHOD

The experiments were all done in the forenoon after at least twelve hours fasting and after one half hour of rest in a semi-reclining position in a comfortable chair. The experiments on the obese patients were performed before reduction diet was fully established. On the day before experiments were to be performed, a general mixed diet of approximately 2500 calories was given. No attempt was made to analyze the work performed in kilogram meters, but the amount of exercise was regulated by fixing the treadmill at a certain number of revolutions per minute so that each patient walked exactly the same distance in seven minutes. Seven minutes was considered sufficient time to allow the oxygen consumption to reach a "steady state" (7 and 8). All the individuals studied performed the exercise at least four times before the experiments were carried out, and the experiments were in every case repeated. After this training a gradual significant decrease in oxygen consumption was not observed when the experiments were repeated. The short period of training (performing the walking exercise on 4 successive days before the experiments were begun), which we instituted, was apparently sufficient to control to a satisfactory degree the training factor in the mild exercise which we studied.

The experiments were carried out as follows. The patient, seated in a comfortable chair on the treadmill, breathed into a 125 liter spirometer for 10 minutes. The expired air collected in these 10 minutes was analyzed to estimate the basal values for oxygen consumption and  $\text{CO}_2$  output. The chair

was then removed and as soon as the patient was comfortably erect the treadmill was set into motion at a speed of 66.7 meters per minute. The change from sitting to standing position was made without detaching the mouthpiece. The expired air during the first two minutes of the exercise was not collected, but the 3rd and 4th, 5th and 6th, and 7th minute samples were collected separately by switching the expired air alternately from one spirometer to another. At the end of the 7 minutes the treadmill was stopped, the chair was replaced and the patient sat comfortably for 40 minutes, during which time all the expired air was collected at intervals of 5 and 10 minutes. All the expired air samples were analyzed in a Haldane apparatus for  $O_2$  and  $CO_2$ . (It was found that the increased oxygen consumption in rest resulting from the previous exercise actually did not extend beyond 20 minutes into the resting period.) The expired air during the first minute following exercise was not obtained so that the oxygen "debt" figures in all cases do not include this minute. During this minute the blood samples were collected.

The output of blood from the heart was determined on another day. The value for the cardiac output was obtained in standing at rest, during exercise (at the end of 7th minute), and 4 minutes after the walking. In the measurement of the cardiac output during exercise a three way valve was used so that it was not necessary to change the mouthpiece in switching from the spirometer to the gas mixture in the balloon. At rest the 2 acetylene samples were collected at the 15th and 20th seconds, in exercise at the 12th and 16th seconds. The expired air and the acetylene mixtures were analyzed in a Haldane apparatus to which had been added a third absorber for acetylene. The values for cardiac output were obtained using the thoroughly reliable acetylene method, as described by Marshall and Grollman (9) and by Grollman (10).

Samples of venous blood were collected at rest and immediately following exercise. In two cases arterial blood was collected before and after exercise. The changes in pH,  $CO_2$  tension ( $pCO_2$ ) and  $CO_2$  content of the arterial samples were noted. The  $CO_2$  content of the arterial blood was measured according to the method of Van Slyke and Stadie (11), the pH according to Hastings and Sendroy (12), and the  $pCO_2$  according to the formula as given by Austin et al (13). Lactic acid values were measured in the venous blood by the method of Hirsch Kaufmann (14), as modified by Lehnartz (15).

By means of the described experiments we observed how the circulatory response in an obese person differed from that in a normal person when each walked 467 meters in 7 minutes at a steady pace. This difference in response could, of course, be considered as due entirely to the extra weight of the obese person, and the disturbances which this extra weight produced. As mentioned above, we had available for study one subject of normal development, rather muscular, and of such size that his weight which was normal for his size, was exactly the same as the weight of one of the obese subjects (case 3). These two were also of approximately the same age (29 and 31), one was a male and the other a female. In these two patients we may assume that the quantity of work performed by each in the walking test was approximately the same. Any marked differences in the circulatory and metabolic responses might be considered as due to superfluous fat.

The essential facts concerning the cases of obesity studied are briefly given

#### PROTOCOLS

*Case 1* F S, a male, was 48 years of age, weighed 104 kgm, and was 166 cm in height. His chief complaint was his extra weight. The patient had occasional palpitation when walking very fast. He suffered from slight edema

about the ankles in the evenings when he had been on his feet a great deal during the day. He complained of moderate dyspnea on exertion, especially when he attempted to blow his horn while marching with a band. He had also noticed increased dyspnea which was definitely related to increasing weight. His mother, two brothers, and three sisters were "very fat." He had been moderately stout (80-85 kilos) for some fifteen years until five years ago when he began gaining considerable weight. He had gained about 25 kilograms in the past five years. This gain was associated with a change in living conditions, at which time there was a definite increase in food consumption, particularly in foods of high fat content.

The fat was symmetrically distributed. There was nothing to suggest a so-called "endogenous obesity." The heart was not enlarged to percussion. There were no murmurs. The second aortic tone was equal in intensity to the second pulmonic, the rhythm was regular, the blood pressure was systolic 130, diastolic 85. The pulse rate over a period of a few weeks was usually 80. There was no edema, the liver was not enlarged, the lungs were clear. Roentgenogram showed a transversely placed heart with a high diaphragm, which was freely movable. The heart was of normal size. The basal metabolism was normal, and the Wasserman reaction was negative.

*Case 2* K. B. was a male, aged 28 years, weighing 96.5 kgm. His height was 163 cm. He complained of obesity, palpitation, nervousness, and dyspnea on exertion. He feared heart trouble. His mother was obese. The patient had been only slightly above average weight since childhood, and the rapid increase in weight had come only in the past six months, during which time he thought he had gained some 10 to 15 kilograms. He had always had an extremely good appetite but gave no satisfactory history as to food intake in connection with the recent gain in weight. The patient was very nervous, easily upset, and frequently trembled all over.

In this case too the fat was symmetrically distributed and there were no signs suggestive of endocrine disturbance. The patient had masculine trichosis. There was no disturbance in water metabolism. The heart exhibited no abnormalities on physical examination. The blood pressure was systolic 140, diastolic 95. There was no edema. The x-ray examination showed a freely movable, high diaphragm, and a heart of normal size which was transversely placed. Electrocardiogram showed only a left axis deviation, of the type considered as being due to position change (16). The basal metabolism was normal. The Wassermann test, blood-film pictures, and urinalysis were negative.

*Case 3* E. W. was a female, 31 years of age, who weighed 88 kgm, and was 158 cm in height. She complained of headaches, palpitation, dyspnea on exertion, general nervousness, weakness, and easy fatiguability. Her mother weighed over 100 kilograms. At 14 the patient weighed 46 kilograms, but at 21 she began to grow stout. Her weight gradually increased. In the past 4 years, since her marriage, there had been a more rapid increase in weight, especially in the face and body. Her forearms and legs were always free from excessive fat. The patient said that her appetite was not particularly good, but that she took a large amount of fluids, especially beer. Palpitation, dyspnea, and fatiguability had gradually increased with increase in weight. The general nervousness, irritability, and headaches seemed definitely associated with a sexual neurosis. No edema was found. Menstrual periods were regular but always slight. The patient had never been pregnant.

The forearms, hands, legs, and feet were relatively free from fat. She had a fat face, thick fat neck, very large breasts and abdomen. The skin was not unusual. The fundus and visual fields were normal. The thyroid was not

TABLE I

Detailed data

Name	Age	Sex	Diagnosis	Height	Weight	Vital capacity	Date	Condition	Respiratory volume	Respiratory rate	Oxygen consumption	Carbon dioxide production	Quotient	Oxygen consumption above basal last 5 minutes	Oxygen debt	Total oxygen requirement	Arteriovenous difference	Minute volume	Stroke volume	Pulse	Blood pressure	Lactic acid	pH	Arterial carbon dioxide per cent	
F. B.	48	M	Obesity	166	104	2700	2/20/31	Sitting	6.56	11	307	250	0.80							89		120/103		7.48	63.0
							2/20/31	Walking	23.0	32	1600	1300	0.81			7080	85	4.0	40.1	89	120/103		7.48	63.0	
							2/21/31	Standing	23.8	32	1600	1300	0.80						12.4	85.6	122	120/103		7.46	64.4
							2/21/31	Walking	25.8	33	1600	1100	0.84						8.9	36.5	110	120/103			
							2/21/31	4 minutes after walking	0.94	33	1460	1100	0.79												
E. W.	31	F	Obesity	168	88	2600	4/9/31	Sitting	6.92	16	281	208	0.74												
							4/9/31	Walking	22.0	28	1190	946	0.82			4810	77	4.1	48.8	81	120/83				
							4/9/31	Standing	20.8	24	1110	840	0.76						11.8	109.0	108	134/84			
							4/9/31	Walking	20.0	28	1100	840	0.76						4.9	52.0	94	125/88			
							4/9/31	4 minutes after walking	8.5	16	360	270	0.75												
E. B.	28	M	Obesity	168	94.5	3400	3/6/31	Sitting	6.9	18	345	260	0.86												
							3/6/31	Walking	20.44	32	1473	1183	0.73			6280	94	3.5	43.8	80	140/103				
							3/6/31	Standing	20.4	32	1473	1183	0.83						12.7	141.0	90	160/103			
							3/6/31	Walking	22.3	33	1480	1200	0.80						4.1	51.3	80	140/103			
							3/6/31	4 minutes after walking	11.1	33	1480	1200	0.79												
E. P.	25	M	Normal	170	68	6000	12/16/30	Sitting	6.54	12	246	218	0.85												
							12/16/30	Walking	13.8	16	802	701	0.87			2800	87	4.3							
							1/14/31	Standing	7.25	14	287	216	0.84						4.0	87.2	70	110/74			
							1/20/31	Walking	14.9	18	770	690	0.86						10.3	128.6	84	120/72			
							1/20/31	4 minutes after walking	7.3	14	261	220	0.88						4.3	58.3	70	110/72			
G. K.	28	M	Normal	180	70	4800	4/17/31	Sitting	6.38	14	240	186	0.83												
							4/17/31	Walking	14.31	18	750	585	0.78			2560	81	3.1	39.8	78	104/74				
							4/20/31	Standing	6.90	16	249	185	0.79						9.2	102.9	90	110/72			
							4/21/31	Walking	16.37	18	790	634	0.81						3.7	65.1	82	105/74			
							4/21/31	4 minutes after walking	8.0	14	268	214	0.80												
H. D.	26	M	Normal	177	78	4350	4/30/31	Sitting	4.74	10	237	187	0.79												
							4/30/31	Walking	17.15	18	811	734	0.88			3510	78	3.08							
							4/30/31	Standing	18.1	16	683	548	0.84						9.7	89.8	80	115/86			
							5/6/31	4 minutes after walking	18.7	16	683	548	0.88												
H. A. (large normal)	29	M	Normal	180	68	5100	4/26/31	Sitting	6.16	12	270	200	0.74												
							4/26/31	Walking	21.24	13	970	740	0.76			3330	75	3.9							
							4/26/31	Standing	8.26	13	251	219	0.78						5.27						
							4/26/31	Walking	20.6	18	800	780	0.88						8.6	110.0	80	119/73			
							4/26/31	4 minutes after walking	8.56	14	310	250	0.81						4.1	66.4	74	114/70			
E. P. (more rapid walking)	25	M	Normal	170	70	6000	5/7/31	Sitting	6.17	12	251	217	0.80												
							5/7/31	Walking	18.5	18	963	850	0.88			4220	90	11.4							
							5/8/31	Standing	19.76	18	1007	870	0.87												
							5/8/31	4 minutes after walking																	

enlarged The heart was not enlarged on percussion No accentuations or murmurs were found The blood pressure was systolic 122, diastolic 84 There was evidence of hypoplasia of the internal genitalia X-ray examination showed a high diaphragm with transversely placed heart of normal size There were no abnormalities about the sella turcica The blood picture and sedimentation rate were normal The glucose tolerance test was normal The basal metabolism was within normal limits Electrocardiogram showed only a left axis deviation of the type associated with transverse displacement of the heart (16)

#### *Vital capacities*

The vital capacities in the cases of obesity were distinctly below normal Using West's (17) formula, we found that the average vital capacity in the three cases of obesity was 25 per cent below normal when calculated according to height standards, and 52 per cent below normal when the surface area standards were used When the corrections which Pratt (18) gives for the values of West were made, the percentage below normal according to surface area standards was not quite so great

In the normal cases these respective values were 9 per cent and 2 per cent The averages are used here and will be used throughout this report for the purpose of comparing the 2 groups when they fairly represent what occurs in the individual cases

#### *Respiratory minute volume*

The respiratory minute volumes at rest showed no significant differences in the two groups During exercise, on the other hand, these respiratory minute volumes were increased to a greater extent in the obese persons This increase was least marked in the obese patient E W whose response was comparable to that of the large normal person H A The average respiratory minute volume in the obese patients during exercise was 28.1 liters, in the normal people, 16.6 liters This may be expected as a result of the difference in respiratory rates If the "dead space" be taken as roundly equivalent to 200 cc, then a person who breathes 32 times a minute into the spirometer is expiring 6.4 liters of air which has not come in contact with the blood, while a person who breathes only 18 times a minute is expiring only 3.6 liters of unused air The individual with the increased respiratory rate would require then for the same alveolar ventilation 2.8 liters of air more in a minute than the individual with the slower respiratory rate The greater respiratory volumes in the obese cases may thus be associated with the differences in respiratory rates which were observed At rest, the differences in respiratory rates were so small as to have no significant effect

#### *Respiratory rates*

The respiratory rates showed distinct differences in the two groups The respiratory rates in the sitting position in the obese patients were 11, 16, and 18, with an average of 15, and in the normal cases the rates

were 12, 14, 10, and 12, with an average of 12. It is of interest to note that whereas in the normal cases the respiratory rates were unchanged in the sitting and standing positions or showed only occasional slight increase on standing, in the obese persons the respiratory rates usually showed a definite slight decrease in the standing position. This is most likely due to the diminution of the upward intra abdominal pressure on the diaphragm in the standing position, thus allowing somewhat more freedom for lung excursions. The average respiratory rate during exercise in the obese patients was 30 per minute, in the normal cases 17. This difference cannot be attributed to the differences in work. In one normal person the work was increased (walking faster) to the point where his oxygen consumption approximated that of the obese patients. Even with this increase in work, the respiratory rate was only 18 per minute.

#### *Oxygen consumption*

The oxygen consumption at rest was slightly greater in the cases of obesity than in the normal cases, but per square meter surface area it was approximately the same. During exercise, however, the oxygen consumption was distinctly greater in the cases of obesity.

Considering simply the oxygen consumptions at rest and during exercise, we find that whereas the average oxygen consumption per square meter surface area at rest in the cases of obesity was 1.48 liter and in the normal case 1.31 liter (difference of 12.68 per cent), it was 7.07 liters in the obese cases in exercise and only 4.42 liters in the normal cases (difference of 60 per cent). When the percentage increase in oxygen consumption in exercise over the resting state is calculated in the two groups, we find that the obese people showed an oxygen percentage increase in exercise of 490 per cent, the normals of 340 per cent, a difference of 150 per cent, calculated according to square meter surface area the increase in the obese cases was 480 per cent, in the normal cases 340 per cent, a difference of 140 per cent.

This greater oxygen consumption is more than can be accounted for simply by the greater work associated with the extra weight or the higher respiratory rates. The average weight of the obese cases was 27 per cent more than the normals, the average total oxygen consumption during exercise 66 per cent greater, and when calculated according to surface area, 60 per cent greater. It is practically impossible to estimate just how much more oxygen was required for the greater number of respiratory movements in the obese patients (19) but it is hardly likely that this was considerable, since the breathing was relatively shallow and not forced. In comparing the normal case with the case of obesity of the same weight and approximately the same age, we find the normal case with an oxygen consumption in exercise of 4.5 liters per square meter surface area, the obese case 6.1 liters, a difference of 33 per cent. While the difference in

sex may account for some of this difference, its chief cause must undoubtedly be attributed to obesity

Hill, Long and Lupton (8) define the oxygen requirement of any given exercise as the total oxygen used during the exercise and in complete recovery from it, in excess of the resting level. In the obese cases this oxygen requirement was distinctly greater than in the normal individuals, averaging 6.37 liters in the obese, and 3.05 liters in the normals. In the obese and normal cases of the same weight the values were 4.81 liters and 3.06 liters respectively.

The oxygen debt in the sense in which Hill, Long and Lupton (8) use the word is determined by measuring the total oxygen used in the recovery period, starting from the end of exercise, and subtracting the oxygen which would have been used in the same period had the body remained throughout at rest. It is assumed that the recovery period in question is sufficiently long to allow a complete return to the resting condition. This represents simply the extent to which the oxygen intake fails to meet the oxygen requirement during exercise, the body thus going into "debt." The oxygen debt may then be taken simply as the difference between oxygen intake and requirement. The oxygen debts in the obese cases were definitely higher than in the normal persons. The average value in the obese was 0.73 liter, in the normal cases 0.48 liter. In the obese and normal cases of the same weight the values were 0.80 liter and 0.35 liter respectively.

#### *Respiratory quotients*

Considerable caution must be exercised in evaluating the respiratory quotients in a study such as this because of their variability, with even slight irregularities in breathing. A particular attempt was made to control this factor. In recent years the numerous studies dealing with the metabolism of glucose and insulin have served to emphasize the many factors which must be considered in a study of the respiratory quotient (20). The changes which we observed, since they were more or less constant, may be mentioned. The respiratory quotients showed a slight tendency to rise during exercise in almost every case, with usually slightly greater rises in the obese individuals, associated with the greater exertion in the walking. Among others Hill, Long and Lupton (21) have shown that such slight increases in respiratory quotient take place during walking experiments.

#### *Arteriovenous difference and cardiac output*

In a study of cardiac output in exercise with the acetylene method, the question of applicability immediately arises. Baumann and Grollman (22) emphasized the fact that in cases in which the cardiac output is greater than 10 liters per minute, the method is not entirely satisfactory. The difficulty is due to the fact that in the minimum length of time

necessary for a satisfactory mixture (alveolar sample) to be obtained by rebreathing in the lung bag system, enough blood has returned to the right heart after a complete cycle to introduce an appreciable error in the arteriovenous difference when the heart output is greater than 10 liters per minute. In a study in which samples from the lung bag system (mixture of acetylene, oxygen, and air) and from the arterial blood were taken simultaneously and analyzed for acetylene content, Grollman, Proger, and Dennig (23) found that even in rapid rebreathing following severe exercise, true alveolar samples were not obtained in less than 10 seconds in carrying out the acetylene procedure. In the present study the first sample in the exercise studies was taken at 12 seconds, so that satisfactory mixture was assured, the second sample was taken at 15-16 seconds. On the basis of the work of Baumann and Grollman (22), in which the right auricle in human beings was punctured *in vivo*, to learn among other things how much and how quickly acetylene returned to the right heart during rebreathing, we may assume that in the cases here recorded, in which during exercise the duration of rebreathing was 15-16 seconds, and in which the minute volumes varied between 10 and 12 liters, approximately 10 per cent acetylene returned to the right heart. This would indicate that the arteriovenous oxygen differences obtained are about 10 per cent too high. Assuming that there were no balancing factors, all the minute volumes in exercise may therefore be considered as approximately 10 per cent too low. (A-V difference is 10 per cent too high and  $M \cdot V = \frac{O_2 \text{ consumption}}{A-V \text{ difference}}$ ) During rebreathing relatively less oxygen is taken up by the blood (quick increase in blood volume in the lungs before the oxygen consumption can be changed significantly) and this relatively smaller oxygen intake, in the blood returning to the right heart, increases the values for the cardiac output. This then serves as a balancing factor so that the error is in reality less than 10 per cent. As a matter of fact, in calculating minute volumes by the acetylene method and comparing them with minute volumes obtained by the Fick principle through direct cardiac punctures in man, Baumann and Grollman (22) found, in the one case in which the minute volume exceeded 10 liters (11.8 liters), that the two values corresponded almost exactly. They suggested that the two sources of error above mentioned probably balanced each other in this case. In any event, in the cases presented here, although the likelihood is that the error is considerably less than 10 per cent, we may assume a maximum possible error of 10 per cent in the cardiac output during exercise. Since this error was always in the same direction, the relative values remain unchanged. Should the errors have occurred only in the higher minute volumes (obese patients) the conclusions that are drawn below would not be altered, evidenced by the fact that an error could only lower the minute volume.



The arteriovenous oxygen differences at rest in the obese cases were slightly higher than in the normal ones, the average being 85 cc oxygen per 1000 cc blood in the obese as compared with 74 cc in the normal persons. Comparing the arteriovenous oxygen differences at rest and during exercise in the three normal individuals (excluding the large normal person) and in the three obese cases, we find that the arteriovenous oxygen differences increased relatively more in the obese cases, in the obese cases, the average of 85 cc increased to 107 cc, and in the normal ones from 74 cc to 84 cc. This might at first glance be taken to indicate a less efficient cardiac response on the part of the obese people. As shown by Dennig and Proger (24), and Bansi and Groscurth (25) cases with cardiac decompensation differ from normal cases in their response to exercise by showing a relatively greater increase in the arteriovenous oxygen difference. Since the consumption of oxygen during exercise in the obese people was, however, so much greater, and since the work performed by them was unquestionably more than that performed by the normal individuals, conclusions can not justifiably be drawn simply from a comparison of the responses of the arteriovenous oxygen differences. When the exercise was increased in the case of one of the normal men to the extent that his oxygen consumption during exercise increased approximately as much over the resting level as in the obese cases, it was found that the response in the arteriovenous oxygen difference corresponded to that seen in the obese cases. In this case the arteriovenous oxygen difference increased from 65 cc to 90 cc. In the normal large person who, in carrying out the exercise, performed approximately the same work as the obese people, the response in the arteriovenous oxygen difference during exercise was as in the obese cases, going from 75 cc per liter of blood at rest to 101 cc during exercise. We find then that the arteriovenous oxygen differences in exercise in the obese people are essentially normal.

It was thought wise to regard measurements of minute volumes in the standing position as basal values in this study chiefly because satisfactory mixing in the lung-bag system is much more difficult to obtain in the obese cases in the sitting position. The work of Grollman (26) indicates that the minute volume does not differ essentially in the two positions.

The average value for the minute volume in the normal cases in the standing resting position was 3.7 liters. In the obese cases it was the same, 3.7 liters. The minute volumes per square meter surface area in the obese cases were slightly lower than in the normals (1.85 liter per square meter surface area in the obese and 1.93 liter in normals).

It follows from what has just been stated in regard to the arteriovenous oxygen differences that the minute volume in the obese people also showed a normal response to exercise  $\left( M V = \frac{O_2 \text{ consumption}}{A-V O_2 \text{ difference}} \right)$ . Thus if the arteriovenous oxygen differences increase normally in relation

to the oxygen consumptions, as was the case in the obese patients, the minute volumes must also increase normally. Since the arteriovenous oxygen difference in all cases increased perceptibly during exercise, the minute volumes did not rise as a simple linear function of the oxygen absorption. We find, in agreement with Grollman (27), that the cardiac output even in light exercise is not a simple linear function of the oxygen consumption. The output of the heart during exercise in the obese cases was therefore normal, or rather, responded as it did in the normal cases. At the end of 4 minutes the cardiac outputs had returned practically to resting levels in all cases.

#### *Pulse rate*

The standing resting pulse rate was found to be higher in the obese cases, being 98, 84, and 80 respectively, than in the normal cases, where they were 70, 78, 82, and 74. In exercise the rates increased in the obese cases to 132, 108, and 90 respectively, in the normals, to 84, 90, 108, and 80. In this instance, a consideration of the average pulse rate in exercise is misleading because in only one of the three cases of obesity did the pulse rate increase markedly during exercise. In the other two cases the increase in pulse rate was similar to that in the normal cases.

#### *Stroke volume*

The average stroke volume of cardiac output was essentially the same in the two groups during rest (obese cases 43.2 cc., normals 46.9 cc.) and during exercise (obese cases 108 cc., normals 112 cc.). Even the obese case which showed such a marked increase in pulse rate during exercise had a stroke volume of 86 cc. while walking.

#### *Blood pressure*

The blood pressure changes in exercise were remarkably constant and similar in all cases studied, obese and normal alike. The systolic pressure was observed to increase slightly during exercise while the diastolic pressure remained practically fixed. This is in agreement with the findings of Bansil and Groscurth (25), and indicates a rather fine quantitative adjustment between increased cardiac output and widening of the peripheral vascular bed since the resistance against which the heart has to work in order to open the aortic valves remains constant during rest and mild exercise.

#### *Lactic acid and other blood changes*

Lichtwitz (28) many years ago found that the lactic acid content of the blood in cases of obesity reached a higher level after exercise than in normal cases. Kugelmann (29), in a study of obese people with no signs of circulatory abnormalities, likewise found higher lactic acid values in obese cases as compared with normal ones after light exercise. In the

two obese cases in this study in which the lactic acid values were obtained before and immediately after exercise, the values almost doubled, whereas in the normal individuals they remained practically unchanged. Even in the case of the normal person whose work was increased to the point where his oxygen consumption corresponded approximately to that of the obese persons, and in the case of the large normal person whose weight approximated that of the obese individuals, the lactic acid values showed no significant changes in exercise.

The  $\text{CO}_2$  content, the  $\text{CO}_2$  tension ( $\text{pCO}_2$ ), and the pH of the arterial blood were calculated in one of the obese cases and compared with the values in one of the normal persons studied. Since no changes were observed in either case it was considered of no particular advantage to make these determinations in every case. These results indicate the mildness of the exercise studied.

#### DISCUSSION

The chief differences between the normal and the obese groups, in which there were no demonstrable pathological changes in the circulatory system, were found in the vital capacities, and in the pulse rate, respiratory rate, lactic acid changes in the blood, oxygen consumption, and oxygen debt on exercise.

The differences which were observed in the vital capacities agree essentially with those which Bowen (30) found in a study of the vital capacity in obesity. Bowen reported that the vital capacity of obese and "overweight" people averages 20 per cent less than normal, when the surface area standard is used, and suggests that the tendency to dyspnea which is so commonly seen in obese people may be accounted for in part by a reduction of the vital capacity. In this regard may be mentioned our observation that the obese persons showed a slight decrease in respiratory rate when standing, obviously due to freer mobility, also that in the one case in which it was attempted, it was impossible in the sitting posture to obtain a true alveolar air sample in the lung-bag system in the same time as in the standing position. This also is undoubtedly due to the mechanical interference with respiration in the obese patient. The diminished vital capacity in obesity may easily be accounted for on the basis of this mechanical disturbance.

In regard to differences in pulse rate, it is well to keep in mind how variable the pulse rate may be, also how totally independent of any primary circulatory disturbance. The study of the response of the pulse rate to exercise is a very unsafe method of estimating cardiac efficiency. As Mackenzie (31) writes, "in perfectly healthy individuals an increased rate in response to effort, and the time which it takes for the rate to return to normal, vary with the individual, the reason evidently being that the excitability of the cells of the sino-auricular node is not a constant

factor amongst normal individuals " It is known that in trained athletes, the pulse rates at rest and in exercise are slower than in untrained individuals (Bainbridge, Bock, and Dill (32)) While it is possible that these differences in pulse rates may in some way be associated with a condition of the heart which affects its responsiveness, it does not, however, seem justifiable to attribute the tachycardia which is seen in obese people to a weakness of the heart. It is possible that purely mechanical disturbances (high diaphragm, increased fat about the heart, etc.) may lead to more rapid and smaller cardiac contractions

As already mentioned, the increased respiratory rate during exercise in the obese cases apparently was not due simply to the greater oxygen consumption, for when the oxygen consumption was increased by increasing the work in one of the normal cases to approximately the same level as in the obese cases, the respiratory rate showed no significant change. The dyspnea which the obese people developed during exercise (average respiratory rate during exercise in the obese cases 30, in the normal cases 17) cannot have been due to circulatory insufficiency, for the cardiac output was found to be normal. There was, furthermore, no evidence of a pulmonary affection which could have been responsible. It is also hardly likely that the slight increase in lactic acid in the blood in the obese cases could have brought about this increase in respiratory rate. Previous experiments on a normal subject (S H P) in which lactic acid increases occurred of the same magnitude as those reported here in the cases of obesity, showed no such relationship between slight increases in the lactic acid of blood, and respiratory rates. As a matter of fact, in an experiment performed on this subject in which, after severe exercise, lactic acid in the blood increased from 8 mgm to 104 mgm per 100 cc., the respiratory rate rose only to 36 per minute, which is just slightly more than the rate observed in the cases of obesity during mild exercise. Experiments in which evidence was presented to support the theory that dyspnea was due to chemical changes within the brain (33, 34), such as increased production of lactic acid, are based on conditions of asphyxia or marked hypo oxygenation of the arterial blood—conditions which did not obtain in the cases presented in this study. It is safe to exclude the chemical changes in the blood as a significant factor in the production of dyspnea. Bansi, Groscurth, and Weigel (35), attribute dyspnea in obesity to some inherent inability to excrete  $\text{CO}_2$ . There results a piling up of  $\text{CO}_2$ , which acts as a respiratory stimulant and is responsible for the increased respiratory rate. If this were true, we should expect to find relatively lower respiratory quotients in the cases of obesity during various stages of exercise. This we did not observe.

An analysis of the respiratory rates and respiratory minute volumes during exercise reveals the fact that the tidal flow is approximately the same in the two groups studied, that, although the rates vary so greatly

there is approximately one liter expired with each breath in both groups of cases. It would appear then that although obese individuals breathe more rapidly than do normal people, they breathe just as deeply. It must be realized, however, that with an increase in respiratory rate, there is normally an increase in the depth of breathing so that a person who breathes 30 times a minute would normally be expected to have a greater tidal flow than a person who breathes only 17 times a minute. Thus when one of the subjects (S H P) increased his exercise to such an extent that he was breathing 28 times a minute, his respiratory minute volume was 53 liters, and his tidal volume about 2 liters or about twice as great as it was when the exercise was such that he breathed only 18 times a minute as in the present study. The obese persons may, therefore, be considered to have distinctly low tidal volumes. Despite indications to the contrary, their breathing is definitely shallow, a fact which must be associated with the lowered vital capacities.

Since rapid, shallow breathing in the obese cases cannot be explained on the basis of primary disturbances in the circulation, lungs, or constituents in the blood, we are left with the assumption that it must be due to mechanical interference with respiration as a result of obesity. It is apparently easier for the obese person to take shallow breaths and more of them than to force the abdominal contents downward to any great extent with each inspiration.

The greater values of lactic acid in the blood in the obese cases during exercise are of interest because, in the absence of liver damage, they suggest circulatory insufficiency. Dresel and Himmelweit (36) found, as did Meakins and Long (37) earlier, that patients with cardiac insufficiency have definitely more lactic acid in the blood after exercise and that the lactic acid values return much more slowly to resting values than in normal people. Theoretically the muscle in cases of cardiac insufficiency is not able sufficiently to resynthesize or oxidize lactic acid resulting from the anoxybiotic breakdown of glycogen. Dresel and Himmelweit think that their method avoids the influence of the liver as a disturbing factor in their results, and also avoids the complication of training as a factor. Eppinger, Kisch, and Schwarz (5), and Meakins and Long (37) also found that after exercise the lactic acid values returned much more slowly to normal in cases of cardiac insufficiency than in cases with no damage of the heart. While one can no longer assume such direct and simple relationships between lactic acid metabolism and cardiac insufficiency as Eppinger, Kisch and Schwarz (5) do in their monograph, still it is true that the comparatively high values of lactic acid which we found in obese cases after exercise indicate definite chemical disturbances in the muscle, which may presumably be associated with cardiac insufficiency.

B. Kugelmann (29) used the technique described by Dresel and Himmelweit in a study of obese persons who, except for obesity, were

in every respect normal. They obtained values for lactic acid which corresponded to the values found by Dresel and Himmelwert in cases with cardiac insufficiency. These increased lactic acid values Kugelmann also found in normal people who for two days before the experiment had been on a carbohydrate-free and protein poor diet—in other words, in whom the glycogen deposits in the body had been materially diminished. Kugelmann presents evidence to show that there is relative insufficiency in the glycogen depots in obese people. He assumes that in obesity the diminution in the carbohydrate depots of the body are responsible for the disturbances in muscle, particularly in lactic acid metabolism. Since lactic acid metabolism is so intimately bound up with the supply of glycogen, his assumptions seem well founded. The high lactic acid values which we obtained, therefore, may be considered as due to disturbed intermediary metabolism with no relationship to circulatory insufficiency.

While it is no longer maintained that an exact simple relationship exists between oxygen debt and the amount of lactic acid present in the blood as first suggested by Hill, Long and Lupton (8), yet it is true that higher values of lactic acid in the blood at the end of short light work predicate higher oxygen debts, for more oxygen is required for resynthesis and oxygenation. The relatively greater oxygen debts which we observed in the cases of obesity are regarded as being due to the greater accumulation of lactic acid in the blood. Since the evidence indicates that excess lactic acid was not due to circulatory insufficiency, it follows that this greater oxygen debt was likewise not associated with any failure of the circulation.

We find then that the differences observed in the two groups studied may all be attributed to chemical and mechanical factors and that there is no evidence of a direct circulatory disturbance or inefficiency. The actual response of the heart to exercise in maintaining an adequate blood volume was normal. The greater increases in arteriovenous oxygen differences in exercise which have been observed in cases of definite cardiac insufficiency were not seen in the cases of obesity presented in this study. It appears then that the signs which may be associated with cardiac insufficiency observed in those cases of obesity which show no organic changes in the circulatory system are not due to an abnormal cardiac insufficiency. Although these cases had clinical signs which might be looked upon as due to inadequate cardiac response (dyspnea, diminished vital capacity, increased oxygen debt), they showed no evidence of abnormal cardiac insufficiency. It is to be remembered that there was no evidence of organic change in the circulatory system, no arteriosclerosis, no hypertension, no cardiac hypertrophy. These are patients then whom we might expect to find, with a return to normal weight, entirely normal individuals.

## SUMMARY AND CONCLUSIONS

1 Various phases of the circulation were studied in a group of obese patients who had no demonstrable pathological changes in the circulatory system, chiefly in their response to mild exercise in the form of walking on a treadmill. The results were compared with the results in a group of normal people.

2 The responses of the cardiac output and arteriovenous oxygen differences to exercise were similar in the two groups.

3 The chief differences between the two groups were found in the vital capacities, and in the pulse rates, respiratory rates, blood lactic acid changes, oxygen consumptions, and oxygen debts in exercise.

4 On the basis of the findings presented, it is believed that the symptoms which are so commonly associated with cardiac insufficiency and which are frequently observed in cases of simple obesity are not due to an inefficient circulatory response, but rather to mechanical and chemical disturbances associated with obesity.

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PROCEEDINGS OF THE TWENTY-FOURTH ANNUAL MEETING OF THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION HELD IN ATLANTIC CITY, N J, MAY 2, 1932

*A Method of Studying Fresh Blood by Dark Field Illumination* By O. C. HANSEN (introduced by H. L. Amoss) Durham, N. Carolina.

The result of five years experience of studying normal and abnormal blood by dark field illumination is given. Comparison is made of this method with that which employs supra-vital stains. Mature and immature leukocytes, as well as red blood cells, are described as viewed by dark field illumination. We believe most, if not all circulating blood cells can be recognized in the dark field and that an accurate impression of haematological disturbances can be arrived at by studying fresh blood in the dark field. It is also a great help in recognizing malaria parasites with ease. The simplicity of this method, as well as the fact that it is not dependent upon dyes and staining technique, should make it available for office practice. Slides of dark field drawings are shown.

*Iron Metabolism Studies* By PAUL REZNIKOFF, New York, N. Y.

The intake and output of iron were studied by the Elvehjem-Kennedy method in two subjects, one normal and one suffering from polycythemia vera, over a period of 8 months and blood counts were followed.

Both subjects approached balance on diets ranging from 10 to 20 mgm iron daily. The normal individual stored iron when given parenteral liver extract, when fed a "secondary" extract, and when given 60 mgm iron as iron ammonium citrate (the equivalent of the iron content of the latter extract). Later in the study neither high iron intake, parenteral liver nor copper sulphate caused any storing of iron. No definite correlation was found between red blood count, hemoglobin and iron balance in this normal individual.

The polycythemic patient showed little response to Fowler's solution or x-ray therapy. Phenylhydrazine caused a fall of erythrocyte count from six to three million and of hemoglobin from one hundred to fifty five per cent, but very little coincident excretion of iron.

These studies, therefore, indicate that the body can store iron for long periods (382.8 mgm in 7 months) without use by the hematopoietic system, or in case of erythrocyte depression (852.4 mgm in 7 months), without excretion, and this work also suggests that iron metabolism should be studied by tissue analysis.

*Treatment of Polycythemia Vera with Solution of Potassium Arsenite* By CLAUDE E. FORKNER (introduced by C. S. Keefer), Boston, Mass.

Arsenic has been used for many years in the treatment of anemia and has been considered an agent favoring regeneration of erythrocytes and hemoglobin. The use of arsenic in polycythemia vera has been reported as either contraindicated or of no value. Türk in 1904, from the result of treating one case with Fowler's solution, concluded the drug was of value. In his case the erythrocyte count was reduced from 9,000,000 to 7,000,000 cells per cu. mm.

Five patients with typical polycythemia vera have been treated by means of oral administration of solution of potassium arsenite. Some of these patients had been followed for several years prior to this treatment. The use of phenylhydrazine hydrochloride and of roentgen rays, applied over the long bones, had been ineffective in one case. The administration of large doses of the pulp of raw spleen had been of no value in two other cases.

Prompt remissions occurred following administration of solution of potassium arsenite in each of these five cases manifested by (1) Lowering of erythrocyte and hemoglobin values to normal or near normal levels, (2) reduction of the leucocyte count to normal or subnormal values, (3) reduction of the hematocrit cell volume, (4) decrease in the size of the spleen and liver, (5) increase in strength and weight, (6) marked subjective improvement.

Remissions may thus be induced and maintained, at least for many months, by continuous administration of the drug.

*Purified Liver Extract Intravenously in the Treatment of Pernicious Anemia*  
By RAPHAEL ISAACS, CYRUS C STURGIS and (by invitation) S M GOLDHAMER and F H BETHELL, Ann Arbor, Mich

It has been possible to remove from commercial liver extract by a very simple method, substances which cause headache, lowering of the blood pressure, chills, fever and vasomotor disturbances. Liver extract so prepared has been given intravenously to 80 patients (400 injections) and the blood counts brought to normal. The extract made from about 100 grams of fresh liver, intravenously, produces a maximum reticulocyte response ten per cent higher than that produced by the extract from 4200 grams given by mouth. Four to six injections of this extract at weekly intervals are sufficient to bring the erythrocyte count to normal, and the maintenance dose is one injection every four to six weeks.

*The Interrelation of Pernicious and Idiopathic Hypochromic Anemia* The Study of a Family in Which Both Conditions Occurred Singly and Combined By CLARK W HEATH (introduced by Dr George R Minot), Boston, Mass

The medical literature reveals evidence to prove an interrelationship between pernicious anemia and idiopathic hypochromic anemia. This is illustrated, not only by reports of hypochromic anemia in families in which pernicious anemia also occurs, but also by reports of hypochromic anemia preceding the onset and during the remission of pernicious anemia.

A family has been studied in which three sisters each had both hypochromic anemia and pernicious anemia with combined system disease. Iron medication in two of these sisters changed an initial microcytosis and low color index to a macrocytosis and high color index. Two brothers had pernicious anemia, one with chronic intermittent diarrhea as the chief symptom, the other with early combined system disease. Another sister died of a progressive anemia resembling pernicious anemia. Two sons of one of the sisters had a macrocytosis without definite anemia and with normal gastric acidity. A daughter of one of the sisters had chronic menorrhagia and hypochromic anemia with hypochlorhydria. Her condition was completely restored to normal by iron. The red blood cells in the anemic members of the family showed an increased resistance span to hypotonic solutions of sodium chloride which became normal as the anemia lessened. There was a high incidence of menorrhagia, migraine and enlargement of the thyroid gland in this family.

The interrelationship of pernicious anemia and idiopathic hypochromic anemia leads to a hypothesis of the etiology of the latter condition. It is believed that in this family there is a hereditary tendency to reduced gastric function. The relationship of pernicious anemia to gastro intestinal disorders has been shown by Castle, who has found that pernicious anemia is secondary to the absence of a specific factor in the stomach secretions. In idiopathic hypochromic anemia there is typically an absence of gastric free hydrochloric acid which may be related to the etiology. It is therefore believed that idiopathic hypochromic anemia is primarily the result of a deficiency, conditioned by a disorder of the gastro-intestinal tract, leading to an inability to absorb or utilize hemoglobin building material from the food.

*Observations on the Etiology and Treatment of the Anemia of Hookworm Disease in Porto Rico* By C P RHODES (by invitation) and WILLIAM B CASTLE, Boston, Mass

Study of over 150 cases of hypochromic anemia associated with hookworm infestation demonstrated that the morphological and physiological characteristics of this anemia are similar to those of other hypochromic anemias without hookworm infection encountered both in Porto Rico and elsewhere, and regarded as mainly due to defective blood formation. No evidence for the presence of substances due to the activity of the hookworm capable of acting either as depressors of blood production or as hemolytic agents was found. Since it was demonstrated that the intramuscular injection of red blood cells into these patients produced increased blood formation, it is logical to assume that the effect of blood loss produced by the hookworm may be significant as a loss of potential hematopoietic factors leading to defective blood formation.

No significant effect upon the anemia was produced during periods of from 2 to 3 weeks and frequently for longer periods either by the elimination of the hookworms or by the administration of a diet enriched by 300 grams of meat and 1500 cc. of milk daily or by a combination of these. On the other hand the daily administration of 6 grams of iron ammonium citrate was almost invariably followed by promptly increased blood formation indicated by reticulocyte crises followed by rapid increases of red blood cell and hemoglobin values and general clinical improvement, despite the persistence of heavy hookworm infestation and the original deficient diet. Substances containing the extract of liver described by Whipple and his associates as of value in hemorrhagic-dietary anemias in the dog were somewhat effective but never as effective as iron in the dosage employed, the liver extract described by Cohn, Minot and their associates as effective in pernicious anemia was found to be without effect. Since in studying these cases of hookworm anemia deficient diets were found to be the rule and gastric anacidity a frequent finding, as is the case in hypochromic anemias not associated with the hookworm, it is suggested that dietary deficiency and gastro-intestinal changes are of major etiological significance.

*Observations on the Etiology and Treatment of Anemia in Pregnancy* By MAURICE B STAUSS (introduced by Henry Jackson Jr.), Boston, Mass

The "physiologic" anemia of pregnancy was investigated by studying the blood, the gastric secretions and the dietary histories of a group of normal women throughout pregnancy. More than half of these women showed a marked decrease or absence of free hydrochloric acid in the gastric juice during pregnancy, with a return to normal following parturition. These women, and those with poor diets, had an average loss of 12 per cent hemoglobin during

pregnancy, whereas women who showed a less marked decrease in free hydrochloric acid and who ate satisfactorily during pregnancy lost an average of only 5 per cent hemoglobin. Three women with permanent post-histamine gastric anacidity had an average loss of 18 per cent hemoglobin in spite of their satisfactory dietary intake during pregnancy. These observations indicate the importance of direct dietary deficiency and deficiency conditioned by changes in the gastric juice in the etiology of the "physiologic" anemia of pregnancy.

Thirty-five women who had less than 45 per cent hemoglobin during the latter half of pregnancy or following parturition were studied. Nineteen of these were found to have post-histamine gastric anacidity and 12 more had little or no free HCl following alcohol test meals.

In addition to this striking incidence of disturbed gastric function, over two-thirds of these patients were found to have had diets which were definitely deficient in iron and other mineral elements and in protein.

None of the 35 patients made any significant improvement during control periods without therapy. Thirty had anemia of the hypochromic type. Liver extract produced no improvement in these cases, but all treated with large doses of iron improved rapidly, gaining an average of 0.65 per cent hemoglobin per day, *whether treated during or after pregnancy*.

The observations on the hypochromic anemia of pregnancy correspond to similar studies on idiopathic hypochromic anemia and indicate that the etiologic factors of direct dietary deficiency and deficiency conditioned by gastric anacidity are common to both. Furthermore, an analogy may be made between the blood requirements of the foetus and the chronic blood loss associated with certain cases of idiopathic hypochromic anemia. In addition, the therapeutic value of iron is shown to be as great in the hypochromic anemia of pregnancy as in other hypochromic anemia.

Five patients had anemia of the hyperchromic type (pernicious anemia of pregnancy). Two of these became well under therapy with iron and two under therapy with liver extract. In view of the observations previously made upon Addisonian pernicious anemia, the effect of beefsteak and gastric juice was studied. Beefsteak alone was found to have no effect upon blood formation upon one patient with macrocytic anemia during pregnancy and to have distinct effect after parturition. Immediately thereafter beefsteak plus normal human gastric juice was found to have a markedly positive effect. Since patients with "pernicious anemia of pregnancy" remain well once cured, it is reasonable to believe in the light of these observations that the anemia is due to the temporary loss of the intrinsic factor of the gastric juice during pregnancy with an ultimate return occurring sooner or later after delivery.

The observations on the hyperchromic anemia of pregnancy indicate that in addition to an iron deficiency in most cases, there is a temporary absence from the gastric secretions of the specific intrinsic factor, the complete absence of which has been invariably observed in Addisonian pernicious anemia in relapse.

Hence, the same etiologic mechanisms hold for the anemias of pregnancy as for similar anemias in nonpregnant individuals, and like therapy is equally efficacious.

*Non-Specific Serological Reactions in Acute Bacterial Infections* By WILLIAM S. TILLET and (by invitation) T. J. ABERNETHY and A. MURRAY FISHER, Baltimore, Md.

The results embodied in this report indicate that sera derived from patients acutely ill with many different acute bacterial infections are capable of reacting with certain strains of *Streptococcus hemolyticus*. Although a mixture of

suitable cultures and reactive sera results in the formation of coarse clumps of bacteria, the phenomenon exhibits certain unique characteristics which differ from the usual specific anti bacterial serological reactions. The unusual features may be summarized as follows:

- 1 Absence of specificity. Sera from patients suffering from infection with pneumococcus, meningococcus, *B. typhosus*, or other bacteria agglutinated in equally high titre the test cultures of hemolytic streptococci.
- 2 The capacity of a patient's serum to react was demonstrable early after abrupt onset of disease, persisted during the active phase, and disappeared rapidly a few days after recovery.
- 3 Agglutinable cultures were rendered inert by heating to the thermal death point.
- 4 The fraction of serum containing the streptococcus reacting factor may be chemically separated from that containing agglutinins specific for the etiological agent.

The results indicate that the phenomenon is a broad reaction to infection induced by different causes and that sera from cases of diverse etiology possess a common, and apparently identical, property. Investigation has therefore been directed toward attempting to identify this property of blood which is acquired and lost so rapidly. The occurrence and general characteristics of the reaction indicate that it may be based upon a mechanism materially different from specific antigen antibody reactions.

Although normal serum fails to react with the cultures, normal plasma causes a coarse clumping of hemolytic streptococci. Plasma from patients acutely ill clump the organisms in higher titre than normal plasma. By mixing organisms contained in several hundred cc. of culture with 1 to 2 cc. of plasma, an actual clot is sometimes formed. There is abundant evidence in the literature that fibrinogen of the blood is increased markedly in acute infections. The results of Maltaner and Johnston and others indicate that "secondary" clots obtained from sera are due to unchanged fibrinogen in the serum. It seems possible, therefore, that the explanation of the hemolytic streptococcus agglutination described depends upon a relationship between the organisms and the excess fibrinogen present in the blood of patients with acute bacterial infections.

*Further Studies on Rheumatoid Arthritis.* By M. H. DAWSON and (by invitation) R. H. BOORS, New York, N. Y.

The studies on rheumatoid arthritis which were presented last year have been continued.

The agglutination phenomenon with *Streptococcus hemolyticus* which was shown to be present in the serum of patients suffering from rheumatoid arthritis has been further analyzed. These studies have revealed that the reaction is highly characteristic both for the bacterial species *Streptococcus hemolyticus* and the disease rheumatoid arthritis. It has been found, however, that certain attributes of the reaction distinguish it from ordinary agglutination reactions occurring during the course of specific infectious diseases.

Studies on the relationship between rheumatoid arthritis and rheumatic fever have been continued. In this connection comparative data on the age and sex incidences of these two diseases will be presented.

The determination of the sedimentation rate of the erythrocytes has been found to be a procedure of great clinical value in the study of cases of rheumatoid arthritis. It has been found that the simple procedure gives a reliable index of the state of activity of the disease and affords an accurate method of determining the results of therapeutic methods instituted.

A large series of patients suffering from rheumatoid arthritis have been treated by the following procedures (a) Intravenous inoculation with *Streptococcus hemolyticus* vaccines (b) Transfusions

The rationale underlying these procedures will be discussed and the results presented

*A Laboratory Method for the Diagnosis of Psittacosis in Man* By T M RIVERS and (by invitation) G P BERRY, New York, N Y

The continued appearance of disease in human beings who have been in contact with parrots and parrakeets has created the necessity for a safe and reliable method for the diagnosis of psittacosis. The following laboratory procedure has, in our experience, met the necessary requirements of safety and accuracy

The patient's sputum, to which 20-50 volumes of meat infusion broth, pH 7.8, and a small amount of alundum have been added, is thoroughly ground in a mortar. The emulsion is centrifuged for 10 minutes at a speed of 3,000 rpm. Then the supernatant fluid is filtered through a Berkefeld V candle at a pressure of 15-30 cm of mercury. Each of 6 mice receive intraperitoneally on 3 successive days 2 cc of the filtrate. The animals are housed in screened battery jars placed in shallow baths of lysol solution in order to prevent the mechanical spread of the infection by insects. All animals are observed for a period of 30 days.

The criteria by which the presence of psittacosis in the inoculated mice is established are (1) the development of illness in some or all of the animals which is usually fatal within 10 days, but occasionally not before 30, (2) the characteristic pathological picture which in mice consists of focal necrotic lesions in the liver and spleen, (3) the absence of ordinary bacterial infections as determined by means of necropsy cultures, (4) the presence in liver and spleen impression smears of the "minute bodies" of psittacosis, (5) the establishment of serial passages of the virus in mice by means of liver and spleen emulsions from the animals receiving the sputum filtrates, and (6) the demonstration in mice surviving the inoculations of sputum for 30 days of an active immunity against a potent strain of psittacosis virus. All of these conditions obviously need not be fulfilled in each instance. Sometimes one, sometimes another serves to establish the diagnosis.

The results obtained by the use of this method in 10 cases of psittacosis are unequivocal demonstration of the virus in 8, probable demonstration in 1, failure of demonstration in 1. Sputa collected from the 5th to the 24th day of disease have been positive. From human autopsy material, e.g., lung, liver, and spleen, the virus of psittacosis has been obtained by means of a similar procedure.

*The Isolation of a New Type of Spotted Fever Virus and Report of a Case*  
By HOBART A REIMANN and (by invitation) HENRY L ULRICH and LUTHER C FISHER, Minneapolis, Minn

The occasion for differential diagnosis between typhus and spotted fever has only recently developed. The widespread distribution of both diseases, render the development of simple methods for differentiation necessary.

A patient in Minnesota presented a clinical picture indistinguishable between typhus and spotted fever. The virus was established by inoculating blood into guinea pigs. The reaction in 140 animals was observed. After intraperitoneal inoculation, fever developed after 5 or 6 days and lasted from 2 to 7 days. Brain virus was less potent. Scrotal swelling appeared in 50

per cent of males. None of the animals died. Rickettsia were found intracellularly in the scrotal exudate. No other important histological changes were noted.

Identification was made by cross immunity tests. Animals immune to Minnesota strain were immune to reinfection with eastern spotted fever but not always to the more virulent Bitterroot strain. Conversely, animals immune to both eastern and Bitterroot strains were immune to Minnesota strain. Animals immune to Minnesota strain were not immune to endemic typhus. Curiously, animals immune to typhus were not regularly non-immune to Minnesota strain. Spotted fever vaccine protected against Minnesota strain. The Minnesota strain is apparently one of exceptionally mild spotted fever.

*The Effect of Giving Digitalis on the Volume Output of the Heart and its Size in Heart Failure* By HAROLD J. STEWART, New York, N. Y.

It appears to be a fact that the giving of digitalis decreases the cardiac output (Burwell, Neighbors and Regen, and Stewart) and cardiac size (Stewart) in normal human beings. This report concerns the effect of its administration when hearts are diseased. In addition to estimations of cardiac output by the acetylene method introduced by Grollman, the size of the heart was measured from x-ray photographs taken at a distance of 2 meters. All observations were made with patients in a basal metabolic state immediately before digitalis was given and at frequent intervals afterward. In all instances digitalis (Merck) 10 gram was given within 24 hours.

We have found that in the presence of heart failure of the congestive type (1) the cardiac output diminishes. (2) With the administration of digitalis if diuresis occurs, (a) the cardiac output increases, (b) the cardiac size diminishes, (c) the ventricular rate decreases and (d) alterations in the form of the T wave of the electrocardiogram occur. (3) Results like these occur both when the rhythm of the heart is normal and in the presence of auricular fibrillation. (4) Even when the only sign of heart failure is dyspnoea, the effects on output and cardiac size like those described, appear. (5) As the effect of digitalis wears off these functions change in the reverse direction.

It appears, therefore, that in heart failure the effect of giving digitalis differs from that in normal persons for instead of diminishing, the cardiac output increases.

*Inequality of Blood Pressure in the Brachial Arteries, with Especial Reference to Disease of the Arch of the Aorta.* By HORACE M. KORN and (by invitation) P. H. GUINAND, Iowa City, Iowa.

It has been tacitly assumed that in normal persons the blood pressure and the volume of the pulse are equal in the right and left brachial and carotid arteries and as a corollary that sphygmie inequality in these arteries is evidence of disease of the aorta, provided that peripheral causes of partial arterial occlusion such as encroachment of the clavicle on the subclavian artery, cervical ribs, thrombosis, embolism and arteriosclerosis, as well as developmental anomalies of the aorta and its main branches, can be excluded. For example, a man aged 45 had syphilitic aortitis and aortic regurgitation, no enlargement of the aorta, dilatation of the innominate artery and equal carotid pulses but a much smaller pulse and lower blood pressure in the right subclavian and its branches than in the left. Extension of the mesarteritis into the innominate and right subclavian, with consequent narrowing of the orifice of the latter, was diagnosed clinically and confirmed postmortem.



Unfortunately, not all cases are so satisfactory. Manifest aneurysm of the aorta is here excluded from consideration, and attention directed to cases which present genuine diagnostic difficulties with respect to etiology and pathological physiology and anatomy. The following case serves as an example. A man, aged 49, presented the usual signs of aortic regurgitation, except that his pulse, although possessed of the requisite volume, exhibited a prominent anacrotic interruption which effectively obscured whatever celerity it might have had. The ascending and transverse portions of the aorta were enlarged. The carotid pulses were equal, but the pulse in the left subclavian and its branches was much smaller than in the right, and the brachial pressure was correspondingly low. After two months of anti-syphilitic treatment the disparity of pulse and pressure was much less pronounced, which seemed to fortify the diagnosis of syphilitic aortitis. Six months later, however, in spite of continued treatment, the disparity had returned to its original magnitude.

The next case which came under observation was even more puzzling. The patient was a man of 33 years who presented the cardinal signs of aortic regurgitation, without dynamic evidence of aortic stenosis, but with an aortic systolic murmur. With the possible exception of this murmur, there was nothing to indicate dilatation or elongation of the aorta. At first, with equal carotid pulses, the left brachial pressure was considerably lower than the right, which suggested syphilitic aortitis at the orifice of the left subclavian artery, and, by inference, a syphilitic etiology for the aortic regurgitation. However, it soon became apparent that the pressure and pulse inequality varied in degree from day to day, and was often wanting entirely. Such variability could not be harmonized with any organic lesion, and because of it a large number of normal persons were examined with the idea of determining the normal incidence of significant inequalities in brachial pressure.

The brachial pressures of 1000 healthy young students were measured with mercury manometers simultaneously in both arms. The results are summarized in the accompanying table (Table 1).

TABLE I  
*Inequalities in pressure levels*

	Maximum systolic		Minimum diastolic		Both levels	
	<i>R &gt; L</i>	<i>L &gt; R</i>	<i>R &gt; L</i>	<i>L &gt; R</i>	<i>R &gt; L</i>	<i>L &gt; R</i>
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
10 mm	6.6	1.6	4.8	3.9	0.5	0.2
11 mm or more	11.8	1.2	7.8	4.2	1.9	0.3
Totals	18.4	2.8	12.6	8.1	2.4	0.5

What we have arbitrarily designated as a significant inequality in brachial systolic or diastolic pressures occurred 419 times, and 310 of these inequalities were occasioned by a higher pressure on the right side. These figures challenge the validity of the old assumption of sphygmie equality in the brachial arteries of normal persons. They also warn against attaching too much significance to inequality of pulse volume and arterial pressure in patients suspected of having disease of the aorta, unless the inequality can be shown to be permanent.

*Direct Stimulation of the Vagus Nerves in Man A Case Report* By HENRY FIELD, JR., and PAUL S BARKER, Ann Arbor, Mich

A man, 40 years of age, presented extreme congestive cardiac failure, apparently due to paroxysmal auricular tachycardia. The heart rate, approximately 190, was not influenced by digitalis, quinidine, or ocular or carotid pressure. Quinine intravenously caused slight transient slowing. The patient became moribund. In a last desperate attempt to stop the tachycardia, the vagus nerves in the neck were exposed surgically and stimulated directly with faradic current. This produced no change in heart rate, although other vagus effects were observed.

Four days later partial atrioventricular block developed, without slowing of the auricles, probably caused by digitalis and quinidine. Rapid improvement followed the ventricular slowing. Now for the first time, ocular pressure and atropine administration revealed evidence of vagal action upon the heart. Later the auricles slowed gradually and sinus arrhythmia appeared suggesting that the rhythm had been sinus tachycardia throughout. For the following six months digitalis was necessary to keep the heart rate below 100.

The case is remarkable for the extreme sinus tachycardia causing failure of an otherwise normal heart, the tachycardia apparently due to complete loss of the cardiac action of the vagi. Furthermore, it is unique as regards the direct electrical stimulation of the vagi.

*An Electrocardiographic Study of Experimental Coronary Occlusion The Inadequacy of the Three Conventional Leads in Recording Certain Characteristic Changes in Action Currents* By FRANCIS CLARK WOOD (by invitation) and CHARLES C WOLFERTH, Philadelphia, Pa.

In dogs, occlusion of the posterior circumflex branch of the left coronary artery regularly produces a deviation of the R-T interval from the iso-electric line in two of the three conventional limb leads of the electrocardiogram. Occlusion of the anterior descending branch produces no such results.

However, if the chest is filled with normal salt solution to insure satisfactory conduction from all parts of the heart, and if a fourth lead is used (electrodes attached to the front and back of the chest), R-T interval changes can be recorded regularly within two minutes after clamping the anterior descending, as well as after clamping the posterior circumflex coronary artery.

When direct heart leads are used, infarctions which are quite small, regularly produce changes in the R-T interval.

These findings suggest that acute myocardial infarction regularly produces a change in the action current of the heart. A deviation of the R-T interval from the iso-electric line is recordable in all instances if the electrodes are suitably placed.

It has been shown in man as well as in the dog that myocardial infarction can occur in such a position in the heart that the characteristic R-T interval deviation will not appear in the routine electrocardiogram, but will appear in the anteroposterior chest lead.

*The Heart in Myxoedema Roentgen Ray Measurements and Electrocardiograms Before and After Therapy* By J LERMAN and R. J CLARK (by invitation) and J H MEANS, Boston, Mass

Zondek, later Fahr have described "myxoedema heart." The condition is one of enlargement which diminishes under thyroid. Coincident changes in electrocardiogram and improvement in cardiac symptoms take place.

We have studied thirty such cases roentgenographically and twenty-four electrocardiographically. Twenty-three showed transverse enlargement varying from 0.5 to 6.3 cm. After thyroid medication the diameter diminished in twenty-seven from 0.3 to 6.9 cm. The shrinkage was gradual and reached its limit in 2 to 5 months. The area of the roentgen heart shadow showed a greater shrinkage than that suggested by the diameter decrease.

The electrocardiograms were all abnormal before thyroid. The most common abnormality was flattening or inversion of the T waves. After treatment most of the electrocardiograms returned to or towards normal. The cases showing the greatest improvement in the electrocardiogram were the ones that showed the greatest shrinkage in the heart shadow.

Under thyroid, high blood pressures tended to drop and low ones to rise. Pulse pressure tended to rise.

Only one patient showed evidence of congestive failure. He died eight months after treatment was begun.

The pathology of the heart in myxoedema in relation to the clinical findings is discussed. The question of pericardial effusion causing these changes is raised.

*Studies on the Function of the Suprarenal Cortex* By G. A. HARROP, JR., and (by invitation) ALBERT WEINSTEIN, Baltimore, Md.

Extensive work has already been reported dealing with the picture of adrenal insufficiency in the dog. The objective physical signs are characteristic as is the rise in the blood nonprotein nitrogen and the diminished excretion of nitrogen and urea in the urine.

The relationship between lipid metabolism and the cortical hormone was studied and it was found that the feeding of certain oils, particularly cotton seed oil, by mouth, enabled suprarenalectomized dogs to carry on normal physiological activities even though the extract supply was only one-third of the minimal maintenance dosage as previously determined. Cholesterol administered alone could not produce this effect. The cholesterol content of the intact adrenal cortex may be increased by the feeding of cholesterol by mouth and is depleted during infections. Injections of large amounts of extract to the intact animal cause a fall within three hours of the blood cholesterol, suggesting some type of inter-reaction between the cholesterol esters and the hormone.

Experiments are in progress in which an attempt is being made to identify this hormone-sparing factor with some particular lipid. The hypothesis is advanced that certain lipoids may serve either as conveyors of the hormone to the cells within which it acts or that they have a specific interaction with it.

*A Case of Generalized Osteitis Fibrosa Cystica (Hyperparathyroidism?) Showing Metabolic Evidence of Spontaneous Healing* By SAMUEL H. BASSETT (introduced by Wm. S. McCann), Rochester, N. Y.

The nitrogen, calcium and phosphorus metabolism has been studied in a woman aged 21 years who presented roentgenological evidence of generalized osteitis fibrosa cystica (hyperparathyroidism?).

The experiment was continued through ten periods of six days each. During the initial thirty-six days, with the patient taking only a constant diet, consisting chiefly of milk, eggs, cheese and orange juice, and containing approximately 111 gram calcium, 141 gram phosphorus, and 10 grams nitrogen per diem, there was a retention of 14.34 grams calcium, 6.96 grams phosphorus and a loss of 7.18 grams nitrogen.

If the assumption be permitted that the calcium stored was deposited in bone as tertiary calcium phosphate, and that a loss of 1 gram of phosphorus occurs with a loss of 17 grams of nitrogen, calculation of the theoretical phosphorus balance from the above data shows the expected retention of phosphorus to be 698 grams. This figure agrees almost exactly with the phosphorus retention demonstrated in this portion of the experiment.

In the seventh and ninth periods, the diet phosphorus was supplemented by the ingestion of a solution of monosodium phosphate. This resulted in a lowering of serum calcium, elevation of serum phosphorus and a marked though temporary increase in retention of both calcium and phosphorus.

*The Action of the Parathyroid Hormone on the Renal Threshold for Phosphorus* By READ ELLSWORTH, Baltimore, Md.

Previous observations of Albright and Ellsworth are confirmed that following the administration of parathormone there is an immediate pouring out of phosphorus in the urine and a fall of serum inorganic phosphorus.

In the present communication the following additional points are made: 1. The phosphorus diuresis following parathormone is quantitatively sufficient to account for the fall of serum phosphorus. 2. Both urine and serum phosphorus changes occur in some experiments at a considerable interval before any observable alteration in serum calcium. 3. By ultrafiltration it is shown<sup>1</sup> that before parathormone is given, the serum inorganic phosphorus is 97 to 100 per cent filtrable. 4. This strongly suggests that one primary action of the parathyroid hormone is to produce a specific lowering of the renal threshold for phosphorus.

*Rickets and Hyperparathyroidism* By BENGT HAMILTON and (by invitation) CHARLES SCHWARTZ, Chicago, Ill.

In a preliminary communication it has been reported that the administration of calcium chloride or calcium gluconate by mouth to rachitic rabbits usually results in death from hypercalcemia, while in normal rabbits a corresponding dose of calcium increases the serum calcium only to a slight degree. It was thought possible that this effect was due to the presence of abnormally large amounts of parathyroid hormone in the blood of rachitic rabbits. A method was devised by which small amounts of parathormone may be demonstrated, using rabbits as test animals. By using this method it was found that the blood of rachitic rabbits is rich in parathormone or some other substance with identical effect on the serum calcium. This finding agrees well with the fact that several investigators have found a hyperplasia of the parathyroid glands in rickets. Our findings indicate that this hyperplasia is accompanied by an increased secretion of hormone. Probably the hyperparathyroidism is secondary to a low calcium absorption which, in the absence of hyperparathyroidism, would lead to a low serum calcium and tetany.

*The Concentration of Electrolytes and Non-Electrolytes in the Serum Following Insulin Administration in Diabetic Patients* By F. W. SUNDERMAN (introduced by J. H. Austin), Philadelphia, Pa.

This study was designed to measure in the diabetic patient the change not only in sugar concentration but also of the other components of serum following the administration of a large dose of insulin. Insulin was withheld in 15 patients suffering with severe diabetes mellitus for a period sufficient to induce marked elevation of the blood sugar. After an overnight fast, blood was re-

moved for analyses and the patients were given from 50 to 150 units of insulin, still fasting. At intervals of 20 minutes, finger prick blood sugar values were followed with a technique which gave us measurements within 18 minutes. When the concentration of blood sugar had fallen to within normal limits or upon the appearance of any symptoms of an insulin reaction, a second specimen of blood was removed by vein for analyses and the patient was returned to his usual regimen.

After the administration of insulin coincident with the fall in serum sugar, cholesterol, and phosphate, the concentration of total base, sodium, and chloride in the serum increased toward normal values. The total osmolar concentration, as measured by the freezing point, decreased somewhat but not below normal. There was no consistent change in the total dry substance. The final picture represents a restoration in general toward normal values.

*The Blood Lactic Acid in Hepatic Disease* By ALBERT M. SNELL and (by invitation) GRACE M. ROTH, Rochester, Minn.

Bollman and Mann have demonstrated elevations of blood lactic acid after experimental liver injury and in hepatectomized animals. The levels of lactic acid in human subjects show comparable changes in the presence of hepatic disease. High values are found in patients with portal cirrhosis in its terminal stages as well as in postoperative hepatic insufficiency. Following surgical procedures on the bile passages, transitory rises are noted. Following hepatic injury from arsphenamine, there was a definite parallelism between the levels of serum bilirubin and those of lactic acid. The relation of these changes in lactic acid values to other laboratory and clinical data is discussed.

*Hypoglycemic Reactions Following Glucose Ingestion* By WALTER S. McCLELLAN and (by invitation) H. D. HALCRO WARDLAW, New York, N. Y.

Symptoms associated with hypoglycemia following the ingestion of glucose have been reported in the literature in 8 patients. During a study of the influence of the previous diet on the metabolism after the ingestion of 100 grams of glucose in the Sage calorimeter, one subject developed symptoms  $4\frac{1}{2}$  hours after taking the glucose on three different occasions. This is the first time, as far as we have found, when the data have been available for analyzing the metabolism preceding an attack.

In the first two observations, when he had been on a high carbohydrate diet, 43 grams of glucose were oxidized showing rapid oxidation before the attack. In the last experiment, preceding which he had received only 25 grams of carbohydrate daily, he utilized only 23 grams for the same period. In this instance the depletion of his blood sugar to 40.8 mgm. was apparently due to a rapid storing of glucose.

Wide variations in the blood sugar time curves of 5 normal controls demonstrated that the previous diet definitely influenced carbohydrate metabolism. In spite of variations in these curves the blood sugar fell to or below its fasting level in all subjects by the end of  $4\frac{1}{2}$  hours. In the patient described above the blood sugar was 40.8 mgm. when he had symptoms. In another man it reached 34.0 mgm. without producing symptoms.

*Thyroid Hyperplasia Produced in Chickens by Ultraviolet Deficiency* By KENNETH B. TURNER and ETHEL M. BENEDICT (by invitation) and ROBERT F. LOEB, New York, N. Y.

Marked thyroid hyperplasia was consistently produced in chickens raised in an environment from which ultraviolet light was excluded by means of an

amber glass filter Rickets was prevented by the daily administration of cod liver oil

In the hyperplastic glands the epithelium was higher, the follicles smaller and more numerous, and the colloid diminished when compared with normal thyroids from control birds. Macroscopically the thyroids of the ultraviolet deficient chickens were greatly enlarged and congested. The average weight of the thyroids from 30 of these chickens was 102 mgm. as compared to an average thyroid weight of 46 mgm. for 28 controls, although the average body weights for each group was the same.

In spite of the evidence of greatly increased activity in the hyperplastic glands, the metabolism as measured by oxygen consumption per minute per gram of chicken determined in four of the group receiving no ultraviolet light was the same as that of four control chickens. The iodine content of the hyperplastic gland was reduced.

The daily administration of potassium iodide to chickens raised in the absence of ultraviolet light effectively prevented thyroid hyperplasia. The thyroids of such birds resembled those of the controls in all details

*The Excretion of Inorganic Sulphates in Man* By J. M. HAYMAN, JR., Cleveland, Ohio

Inorganic sulphates were estimated in the blood by Power's and Wakefield's method, and the urine by Fiske's benzidine method. Inorganic sulphates are less concentrated by the human kidney than creatinine, and usually less concentrated than urea. In accordance with the belief in filtration and reabsorption, this is regarded as evidence that some sulphate diffuses back through the tubule cells. After intravenous injection of sodium sulphate, the concentration ratio approaches that of creatinine. In nephritis with elevated serum sulphate, the excretion resembles that in a normal individual after injection of sulphate.

*Complement Fixation in Smallpox and Vaccinia* By R. F. PARKER and R. S. MUCKENFUSS (by invitation) and D. P. BARR, St. Louis, Mo.

Complement fixation has been positive in twenty six cases of smallpox in which the fluid was obtained before the ninth day of the eruption, using serum of rabbits hyper-immunized to vaccinia, and three hour ice box fixation with a capillary drop of vesicle fluid as antigen. After this time some negative reactions were observed. Fluid from the pustules of primary vaccinations gave positive reactions in all cases studied.

The blood of patients was studied by this technique, using ether extracted testicular vaccinia virus as antigen. The reaction became positive about the seventh day of smallpox.

*Studies on Histamine in the Gastric Juice* By CHARLES L. BROWN (introduced by Cyrus C. Sturgis), Ann Arbor, Mich.

This study is an investigation of one phase of the fate of histamine in the body, namely, whether histamine is secreted by the stomach. Histamine has been isolated from the gastric mucosa, but report of previous work to investigate its possible presence in the gastric secretion has not been found.

The methods of extraction of histamine used by Best in the study of tissues were applied to gastric juice. The gastric juice extracts were assayed by the blood pressure method, using cats, as recommended by Best.

Twenty-seven samples of gastric juice, representing juice obtained with and without histamine stimulation, in normal and diseased individuals, were studied. Twenty-five of these showed a measureable amount (by assay) of a

blood pressure depressing substance which resembled histamine in its evanescent effect. Normals without histamine stimulation assayed from "not potent" to 0.0535 mgm (in terms of histamine base) histamine activity per 100 cc gastric juice, normals with histamine stimulation assayed from 0.0023 to 0.0411 mgm (in terms of histamine base) histamine activity per 100 cc gastric juice.

These gastric juice extracts in very great dilution caused violent contraction of virgin guinea pig uterus.

Two samples, each made up of two liters of gastric juice, were processed to isolate histamine in the form of dipicrate, but no crystals were obtained which conformed to the melting point and crystallographic characteristics of histamine dipicrate. However, the final extracts were potent by the assay method. The very small possible quantity of histamine may account for the inability to obtain the crystals.

*Conclusion* A histamine-like substance, having an evanescent blood pressure depressing effect and causing violent contraction of the virgin guinea pig uterus, is found in the gastric juice, when the juice is obtained without or with histamine stimulation.

*The Electrocardiographic Changes Following Ligation of the Smaller Branches of the Coronary Arteries in the Dog* By W. M. FOWLER and H. W. RATHE (by invitation) and FRED M. SMITH, Iowa City, Iowa.

The object of this investigation was to determine the effect of ligation of the smaller subdivisions of the coronary arteries on the electrocardiogram and to correlate these electrocardiographic changes with the location and character of the myocardial lesion.

Thirty-seven experiments were performed upon twenty-four dogs. Two of the dogs died from pneumonia, the remaining animals were sacrificed at varying intervals following the operation, and pathological studies made of the myocardial lesion.

The dogs were anaesthetized with ether, a tracheal canula introduced, and the anaesthetic continued under positive pressure. An incision was made through the chest wall on either the right or left side of the sternum, depending on the ventricle to be exposed. A control electrocardiogram was taken prior to the anaesthetic. Subsequent curves were taken eighteen hours following the operation and at daily intervals thereafter. In the first experiments, curves were taken at frequent intervals immediately after the operation, but this was discontinued, since in every instance the significant electrocardiographic alterations persisted for more than eighteen hours. Furthermore, premature contractions were often so numerous during the early post-operative period that the character of the T deflection was obscured.

A negative T deflection in one or more leads was the most distinctive finding. This was not necessarily accompanied by alterations in the R-T segment. In the subsequent course, the T wave became less negative and in from three to five days, returned to a positive phase. This was followed in many instances by an increase in the amplitude of this deflection and a later return to the original level. When the heart was examined after the electrocardiogram had gone through the successive alterations, a small fibrotic area was found in the myocardium below the ligature. If, however, the heart was examined during the early electrocardiographic alterations, a small area of degeneration with round cell infiltration was noted.

Vessels were ligated on the anterior and posterior surfaces of the left ventricle. Electrocardiographic changes occurred in most instances although no

correlation could be found between these alterations and the location of the occlusion. A vessel of the right ventricle was ligated in seven dogs. In six instances the ligation was followed by an alteration in the electrocardiogram.

In seven dogs the pericardium was opened and sutured without disturbing the myocardium. The electrocardiographic changes were identical with those following ligation of the smaller vessels. When the hearts were examined at necropsy there were either adhesions between the epicardium and pericardium at the site of the incision or a discoloration of the underlying epicardium. The superficial vessels were patent, but on microscopic examination, degenerative changes of the muscle fibers immediately under the epicardium with a slight degree of round cell infiltration was found.

These experiments indicate that minute myocardial damage is sufficient to produce significant alterations in the T deflection of the electrocardiogram. The early changes are associated with an acute degenerative process in the myocardium. A return of the electrocardiogram to normal is accompanied by a replacement of the areas of degeneration by fibrous tissue.

*The Effect of Thyroxin upon the Metabolism of Isolated Normal and Malignant Tissue* By OVID O MEYER and CLAIRE McTIERNAN (by invitation) and JOSEPH C AUB, Boston, Mass

By the method of Warburg, micro respiration measurements were made on isolated tissues. Mouse sarcoma C. R. 180 served as the neoplastic tissue and liver from the same mouse was used as the normal tissue control in each experiment. It was found that after daily subcutaneous injections of thyroxin for periods of three to thirty three days the metabolism of the liver was appreciably elevated, whereas the oxygen consumption of the tumor in the majority of cases decreased to a significant degree when compared with control animals. The tumors from mice that had received thyroxin were similar microscopically to the controls.

To eliminate the possibility that the failure to obtain the usual thyroxin effect was due to the tumor's lack of innervation, thyroxin was administered to dogs in 28 to 44 mgm. doses over periods ranging from nine to thirteen days. At the end of the given period, the dogs were killed and the metabolism of the cortex of the denervated and normal kidneys was measured and found to be similar.

**Conclusion** Thyroxin failed to elevate the tumor metabolism in a manner similar to that of normal tissue. This is probably due to some factor within the tumor tissue rather than a lack of nerve supply.

The effects of pancreatectomy on normal and tumor metabolism was also briefly discussed.

*The Effects of Temperature and of Tissue Turgor on the Movement of Fluid through the Human Capillary Wall* By EUGENE M LANDIS and (by invitation) JOHN H GIBBON, JR., Philadelphia, Pa

A plethysmograph measuring the volume of a segment of forearm under a pressure of 200 mm Hg was used to study certain factors concerned in the movement of fluid through the capillary wall. Measurements made by means of the pressure plethysmograph are relatively independent of the variations in arm volume produced by vasomotor changes. The temperature of the apparatus was kept constant to within one degree Centigrade by circulating water through the space between the double wall of the instrument. The subjects were recumbent, with one arm abducted and the forearm extending vertically.



Tissue fluid accumulates at very low venous pressures, the rate of accumulation being proportional to venous pressures at least up to 60 cm water. The temperature of the forearm conspicuously influences the movement of fluid. The rate of filtration produced by a venous pressure of 60 cm water with a forearm temperature of 44.5° C is over twice as great as the rate observed at the same venous pressure and a forearm temperature of 14.5° C.

When volume determinations are repeated over an extended period the rate of filtration produced by any given venous pressure gradually decreases as fluid accumulates in the tissue spaces. This occurs at low and high venous pressures indicating that tissue turgor is a factor in preventing the loss of large amounts of fluid from the blood stream.

*The Rates of Utilization of Thyroxin and of Desiccated Thyroid in Man: Relation Between Iodine in Desiccated Thyroid and Thyroxin* By WIL-LARD OWEN THOMPSON and (by invitation) LAWRENCE L. McLELLAN, Chicago, Ill

These observations were made to determine the rate of production of the thyroid hormone in man. We have found that the minimum amount of thyroxin which must be injected intravenously or subcutaneously *every day* in order to maintain the basal metabolism of patients with marked myxedema at the standard normal level is 0.3 mgm to 0.4 mgm. (This figure has not been determined before by daily injections.) The basal metabolism in these patients before treatment was minus 40 to minus 45 per cent and, therefore, they had little or no functioning thyroid tissue. The following observations suggest that most of the thyroxin was utilized by the body to increase its caloric expenditure and not excreted unused.

1. Injecting 1.5 mgm every fifth day had the same effect as injecting 0.3 mgm every day.

2. The intravenous injection of a single dose of 10 mgm of thyroxin in a patient with marked myxedema produced roughly the same number of excess calories per milligram of thyroxin as injecting the same amount of thyroxin in divided doses of 0.3 mgm per day.

Observations have been made which make it appear probable that when thyroxin is injected into an individual whose thyroid function is normal to begin with, much of it is promptly excreted unused. However, it would seem that a dose of thyroxin which is just adequate to supply a deficiency in the body may be used almost completely.

In patients with myxedema the calorogenic effects of thyroxin injected intravenously and desiccated thyroid administered by mouth are the same on the basis of their iodine contents. In a patient who required 0.3 mgm of thyroxin injected intravenously every day to maintain the basal metabolism at the standard normal level, it was necessary to administer 15 grains (100 mgm) of desiccated thyroid daily by mouth to accomplish the same result. This amount of desiccated thyroid contained approximately 0.2 mgm of iodine, the amount contained in 0.3 mgm of thyroxin. It would thus appear that all the iodine in desiccated thyroid is in a form equivalent to thyroxin from the standpoint of its physiological effects, and that, when the dose is just adequate to replace a deficiency in the body, it is absorbed almost completely from the gastro-intestinal tract.

*Proof of Direct Communication Between Coronary Arteries and the Chambers of the Heart* By JOSEPH T WEARN and (by invitation) T G KLUMPF and L. J ZSCHIESCHE, Cleveland, Ohio

It was pointed out in 1928, as a result of perfusion experiments, that perfusate introduced into the coronary arteries escaped into the chambers of the heart without passage through the capillaries. The existence of these channels has been denied by Anrep, Grant and Viko and Stella. Recent injection experiments, which consisted of injecting a substance into the coronary arteries too thick to pass through the capillaries and finding the same substance emerging from the Thebesian vessels, give further evidence of the existence of Thebesian communications with the arteries. Serial sections through one of these openings prove conclusively that there is direct connection between coronary arteries and the chambers of the heart. The importance of these communications is obvious when one considers the frequent obstruction of the coronary arteries without coronary infarction.

These vessels have not been described heretofore.

*High Calcium Low Phosphorus Diets for the Rat Long Time Metabolism Studies* By ALFRED T SHOHL and (by invitation) HELEN B BROWN, EDNA E. CHAPMAN, CATHERINE S ROSE, and ESTHER SAUERWEIN

Short time metabolism studies have shown that retentions high in calcium and low in phosphorus result from these diets. Does this type of retention continue? If so, is the body composition altered? When cod liver oil is added to the rickets producing diet of Steenbock, rats continue in good nutrition for a long time. Their growth is restricted by the phosphorus (law of the minimum) and the high calcium, low phosphorus retentions are still present after 20 weeks on the diet. The body composition is not altered and the bones are of normal composition.

*The Rôle of the Pulmonary Circulation in the Dyspnoea of Circulatory Failure and of Hyperthyroidism* By GEORGE P ROBB (by invitation) and SOMA WEISS, Boston, Mass

Although the velocity and volume of blood flow are increased in hyperthyroidism and decreased in circulatory failure, yet in both conditions the patients exhibit weakness, a tendency to dyspnoea, orthopnoea and a low vital capacity. This similar clinical behavior of patients with opposite disturbances of the blood flow has not so far received satisfactory and detailed explanation. Simultaneous quantitative study of the pulmonary circulation and of the ventilatory function of the lungs has, however, thrown light on the mechanism underlying this clinical paradox.

A group of patients suffering from hypertensive and luetic heart disease had dyspnoea and orthopnoea and a retarded pulmonary blood flow but gave no evidence of congestive failure in the periphery, the velocity and volume of the blood flow and the peripheral venous pressure being normal. However, although the amount of blood in the lungs was normal or slightly increased the residual air was absolutely or relatively increased and the vital capacity was low. The index between the vital capacity and the residual air was particularly decreased. In the more severe cases of dyspnoea and orthopnoea, the amount of blood in the lungs was often considerably increased during periods when the peripheral circulation still showed no signs of congestive failure. The ratio between the vital capacity and the residual air was considerably disturbed in such cases. With clinical improvement due to rest or

digitalis both the pulmonary circulation and the dynamics of the lungs approached the normal state

In a group of patients suffering from hyperthyroidism it was also observed that the actual amount of blood in the lungs was normal or increased and the velocity and volume of the blood flow in the pulmonary circuit was increased. The residual air space in these, as in the cardiac patients, was increased and the vital capacity was decreased. The ratio between the vital capacity and the residual air was decreased. Following the administration of Lugol's solution, and after thyroidectomy, the circulation and ventilation of the lungs returned to normal.

From these observations we have concluded that in both the cardiac and hyperthyroid patients, an increased capillary pressure with or without engorgement within the pulmonary circuit produces a functional emphysema of the lungs with a stiffening of the alveoli and a resulting low vital capacity. In heart disease, this increased capillary pressure is the result of back pressure from the impaired heart, in hyperthyroidism, it may develop either from active dilatation of the arterioles or from a passive dilatation due, again, to back pressure from the heart. Gross encroachment on the alveolar space by blood is not an essential factor in the development of the functional emphysema or low vital capacity in circulatory failure or in hyperthyroidism. The response of the lungs with increased capillary pressure and secondary functional emphysema in both conditions is to a large extent the explanation of the identical clinical manifestations discussed.

This study demonstrates the clinical significance of a quantitative, correlated observation of the pulmonary circulation and the ventilatory function of the lungs. It also demonstrates that disturbances in the pulmonary circulation and ventilatory mechanism of the lungs may develop independently of the state of the peripheral circulation.

*The Equilibrium between Cerebrospinal Fluid and Blood Plasma VII The Distribution of Sodium and of Chloride Ions between Cerebrospinal Fluid and Blood Plasma and the Donnan Membrane Equilibrium* By FRANK FREMONT-SMITH and (by invitation) MARY ELIZABETH DAILEY, Boston, Mass

We have previously shown that the human cerebrospinal fluid is in osmotic equilibrium with the blood plasma. Since the cerebrospinal fluid is almost protein-free, it is of interest to study the distribution of electrolytes between this fluid and plasma to determine to what extent this distribution may be explained by the laws of thermodynamics. During the past eight years we have studied the distribution of protein and of chloride between human blood plasma and cerebrospinal fluid in over 360 instances. In over 100 of these cases the distribution of sodium has also been determined. The total osmotic pressure has been determined by the freezing point method, and the water content by drying to constant weight.

In 25 instances in which the plasma and cerebrospinal fluid were shown to be in equilibrium by freezing point determinations, and in which the cerebrospinal fluid was essentially normal in composition, the ratio of spinal fluid sodium to serum sodium averaged 1.03 while the ratio of serum chloride to spinal fluid chloride averaged 0.82. If the Donnan membrane equilibrium governs the distribution of ions between these two fluids, the sodium in the water of serum should be greater than the sodium in the water of cerebrospinal fluid, whereas the chloride in water of the cerebrospinal fluid should be greater than that in the water of serum. This is shown to be the case when the con-

centration of these ions in water content of serum and cerebrospinal fluid is calculated. The average sodium content of serum water is 337 mgm per 100 cc., in spinal fluid water 324 mgm. per 100 cc., the chloride in serum water 386 mgm per 100 cc., and in cerebrospinal fluid water 438 mgm per 100 cc.

If sodium and chloride is all present as diffusible ions, the distribution ratios for sodium and chloride in the water of these two fluids should be identical according to the formula

$$r = \frac{[\text{Na}]_{\text{O.S.F.}}}{[\text{Na}]_s} = \frac{[\text{CL}]_s}{[\text{CL}]_{\text{O.S.F.}}}$$

The ratios determined in water content, average

$$\frac{\text{cerebrospinal fluid sodium}}{\text{serum sodium}} = 0.96$$

and

$$\frac{\text{serum chloride}}{\text{cerebrospinal fluid chloride}} = 0.88$$

By means of the formula derived by Van Slyke, Wu and McLean (J Biol Chem, 1923, lvi, 765)

$$r = \frac{[\text{BP}]_s + \sqrt{[\text{BP}]_s^2 + 4[\text{A}]_s([\text{A}]_s + [\text{BP}]_s)}}{2([\text{A}]_s + [\text{BP}]_s)} = 0.93$$

we have calculated the theoretical value for the Donnan ratio to be 0.93. It will be seen that this value is almost midway between the determined ratios for sodium and for chloride in water content. It is possible that some of the water of serum is bound to protein or to other colloids, and is therefore not available for the solution of ions. If it is assumed that an average of 4.3 cc. of water per 100 cc. of plasma is so bound leaving 89.3 cc. of "free water" per 100 cc of plasma, the distribution ratio for sodium now becomes identical with that for chloride and equals 0.92.

The agreement between this value and the theoretical ratio (0.93) is extraordinarily good considering the complexities of the two fluids studied and the necessary assumptions involved. Moreover slight variations in the composition of the plasma are constantly taking place which would tend to disturb the equilibrium.

**Conclusion.** From a study of the distribution of sodium and chloride between human blood plasma and cerebrospinal fluid it has been shown that the distribution of these ions is in general in agreement with the Donnan membrane equilibrium although quantitative identity between the observed and calculated ratios has not been obtained.

*The Circulation of the Brain and of the Leg in Man as Effected by Alterations in the Gaseous Content of Arterial Blood* By WILLIAM G. LENNOX and (by invitation) ERNA LEONHARDT, Boston, Mass.

Fifty experiments were conducted in which patients breathed various mixtures of  $\text{CO}_2$  and of  $\text{O}_2$ . Before and during the procedure blood was drawn from an internal jugular and a femoral vein and from an artery. The  $\text{CO}_2$  content and the  $\text{O}_2$  content and capacity of these samples was measured.

For the brain, an increase in the  $\text{CO}_2$  content of the arterial blood resulted in a marked decrease, and a loss of  $\text{CO}_2$  in a marked increase, in the A-V difference. Alteration of the  $\text{O}_2$  saturation of arterial blood produced less distinct

changes in the opposite direction. An increased saturation was associated with an increased, and anoxemia with a decreased A-V difference. These observations are believed to indicate that an increase of  $\text{CO}_2$  or a decrease of  $\text{O}_2$  in the arterial blood causes a dilatation of cerebral vessels, and the opposite conditions a constriction. These conclusions are consistent with the direct observations of pial vessels of the cat reported by Wolff and Lennox.

Circulatory changes in the leg did not parallel those in the brain.

*The Effect of Rattlesnake Venom on Blood Sugar* By G O BROWN, St Louis, Mo

Lethal doses of the venom of *Crotalus atrox* gives rise to an antemortem hyperglycemia. Sublethal doses at times show the same effect, although not so regularly as the lethal doses. The causation of this hyperglycemia is considered together with the effect of insulin and antivenom on the increased level of blood sugar.

*Two Mechanisms in the Production of Duroziez's Sign, their Diagnostic Significance and a Clinical Test for Differentiating between Them* By HERRMAN L. BLUMGART and A. CARLTON ERNSTENE, Boston, Mass

*The Mechanism of Vomiting Induced by Quinidine* By A. CARLTON ERNSTENE and (by invitation) SAMUEL LOWIS, Boston, Mass

Since knowledge of the mechanism of the toxic symptoms of a drug is of importance in clinical therapeutics, we have studied the mechanism of emesis induced by quinidine. Contrary to expectation, the results of the investigation indicate that quinidine does not cause vomiting reflexly by stimulating afferent fibres from the heart. Quinidine emesis is mainly of peripheral origin, however, the afferent impulses reaching the medulla chiefly by way of the sympathetic division of the autonomic nervous system. We have been led to this conclusion by the following facts established in experiments on cats in which the effective vomiting dose of quinidine had been determined previously by intramuscular injection.

Denervation of the heart according to the technique of Cannon, Lewis and Britton failed to prevent nausea or vomiting in eleven of thirteen instances. Solutions of quinidine sulphate did not cause emesis when applied to the region of the vomiting center in the medulla. Removal of the stomach and intestinal tract did not prevent emesis, if the animals were in good postoperative condition. Paralysis of the vagus nerves by atropine sulphate did not prevent vomiting, although somewhat larger doses of quinidine frequently were necessary to induce emesis after atropine. Nicotine invariably prevented vomiting, and ergotoxine usually did so. Section of the spinal cord at the level of the second thoracic vertebra prevented emesis except at times after greatly increased amounts of quinidine.

The results of the investigation indicate that the chief sites of the therapeutic and toxic actions of quinidine are entirely different.

*The Effect of Experimentally Produced Liver Damage on the Blood Fat Curve Following Epinephrine* By CHESTER M. JONES and (by invitation) JOSEPHINE W. FISH, Boston, Mass

Elsewhere proof has been given of variations in the blood fat curve occurring in individuals suffering from various forms of liver disease. We have tried to reproduce these findings in animals with experimentally produced liver damage.

Chloroform and coal tar have been used in different doses to produce varying degrees of liver necrosis in rabbits. As in human beings the blood fats in normal rabbits rise abruptly following the subcutaneous injection of epinephrine. Following the use of chloroform or coal tar, with the resulting hepatic necrosis, the shape of the fat curve has been substantially modified in these animals. In animals with seriously damaged livers the curve goes progressively downwards. Apparently with somewhat less severe damage the fat curve tends to be absolutely level.

These variations from the normal rise and fall are identical with curves observed in human beings with very serious degrees of liver damage and strengthen our conviction that marked variations from normal in the behavior of the blood fats after the injection of epinephrine are of distinct prognostic value.

*The Postoperative Disturbance in the Respiratory Mechanism* By W J MERLE SCOTT, Rochester, N Y

After operation there often is a marked disturbance in the respiratory mechanism as shown by the volume of the lung and particularly by a reduction in the vital capacity. A general parallelism is found between the latter and the incidence of postoperative pulmonary complications for the different areas of the body. The factors responsible for the diminution in vital capacity have been studied. Mechanical factors such as the alteration in intra-abdominal tension are relatively unimportant. Afferent stimuli from the wound appear to be chiefly responsible for it. After the reduced vital capacity is established, hyperventilation causes little change in it but interruption of pain from the wound results in a considerable increase. If the wound can be made entirely anesthetic for forty-eight hours after operation, most of the decrease in vital capacity can probably be prevented and a coincident reduction in the incidence of postoperative pulmonary complications achieved.

*Parathyroid Poisoning as a Complication of Hyperparathyroidism in Man* By FULLER ALBRIGHT, Boston, Mass

Simple hyperparathyroidism leads to skeletal decalcification, and even death from insufficiency of a bony skeleton. Collip has shown that very large doses of parathyroid extract in animals lead to a very different and sudden type of death. Hueper has shown that this death is associated with calcium deposits in certain abnormal places. Evidence is here presented that there is an increased calcium deposit in tissues where calcium is normally deposited. This strengthens the hypothesis that the calcium deposits are the result of an oversaturation of the blood with calcium phosphate.

Over fifty cases of hyperparathyroidism are now in the literature. One of three cases is cited where the manner of death and the autopsy findings not only make the diagnosis of hyperparathyroidism, but also that of parathyroid poisoning. The importance of recognizing the existence of this fatal complication is in its possible prevention. The series of events which lead up to it is discussed.

*The Blood Iodine in Thyroid Disease* By GEORGE M CURTIS and (by invitation) CHESTER B DAVIS, Chicago, Ill

The constant occurrence of iodine in human blood is now established. Our studies indicate a relationship between thyroid activity and the blood iodine level. There is thus opened up a new approach to studies of thyroid function in health and disease.

The normal iodine content of human blood in this region is 123 gamma per cent. One gamma equals 0.001 mgm. This is elevated in patients with toxic goiter to over twice the normal value, and more so in toxicity associated with diffuse hyperplastic than with nodular goiters. It rises sharply following preoperative iodization, and falls following the thyroidectomy. After adequate thyroidectomy, it remains at a low normal level.

In hypothyroid states the blood iodine is lower than normal. This is true for cretinism, diffuse juvenile goiter with hypothyroidism, and postoperative myxedema of varying degree. The administration of desiccated thyroid, to patients with postoperative hypothyroidism, results in a definite and early rise of the blood iodine, which subsequently falls. Patients maintained upon desiccated thyroid over long periods maintain a high normal blood iodine. Members of the same family with varying manifestations of thyroid disease show striking variations in the blood iodine level, although living under similar conditions and on the same food and water.

*The Nature of the Cardiac Enlargement Resulting from the Administration of Active Thyroid Material* By FRANCIS M. SMITH (by invitation) and EATON M. MACKAY, San Diego, Calif.

The increase in the weight of the heart which follows the administration of desiccated thyroid to the albino rat bears a linear relationship to the basal metabolic rate and the heart weight as varied in this manner is directly proportional to the total metabolism as measured indirectly by the food intake. In light of the relationship known to exist between the work of the heart as expressed by the cardiac output and the basal and total metabolism it is suggested that the cardiac enlargement which follows thyroid intoxication is in the nature of a simple work hypertrophy. The chief objection to this view is the accumulation of lactic acid in such a myocardium. The possibility exists that this is akin to the accumulation of lactic acid during a given effort stimulus in untrained skeletal muscle. If so it might be expected to disappear after long continued maintenance of a given thyrotoxic level or possibly through the breathing of oxygen rich air.

*The Relationship between Oxygen Consumption and Nitrogenous Metabolism* By C. W. BALDRIDGE, Iowa City, Iowa

As previously demonstrated, the rapid production of erythrocytes during induced remissions in pernicious anemia is associated with a decreased oxygen consumption. We also confirmed the work of Riddle and Sturgis and of Krantz and Riddle, who found increases in total oxygen consumption coincident with the destruction of leukemic leucocytes by roentgen ray.

Further investigations have revealed the following relations: 1. Increased blood destruction in polycythemia occasioned by phenylhydrazine is associated with increased oxygen consumption. 2. Rapid erythropoiesis following hemorrhage in dogs is associated with a slightly decreased total oxygen consumption. 3. Transfusion of compatible blood in dogs is followed by a rise in oxygen consumption which is delayed and coincides with the disappearance of the transfused blood. 4. Subcutaneous injection of hemolyzed blood in dogs is associated with an immediate rise in oxygen consumption. 5. In phlorhizin diabetes and in the period of nitrogen retention which follows it, the oxygen consumption curve is approximately a mirror image of the nitrogen balance curve. This corresponds with the findings in blood dyscrasias but the changes are of greater magnitude.

From these and other observations the following tentative generalizations are drawn 1 Nitrogen retention (cell formation) is associated with a decreased oxygen consumption 2. Nitrogen loss (cell destruction) is associated with an increased oxygen consumption

*The Significance of the Mercury Combining Power of Blood* By P S HENCH, Rochester, Minn

The mercury combining power of blood is an index of its urea content and the amount of urea in the blood can be determined simply by an estimation of its mercury combining power The technic of the test is so simple that it can be carried out with a minimum of laboratory equipment, and it can be completed in fifteen minutes This test is an extension of the work done some years ago on the salivary index (the mercury combining power of saliva), and a review of the recent work by the author and others, confirming the accuracy and value of the test, is presented.

*Basal Metabolism Changes During the Dietary Correction of Under-Nutrition*  
By FRANK A EVANS and (by invitation) JAMES M STRANG, Pittsburgh, Pa.

Studies of the basal metabolism have been made on 11 patients with severe under nutrition These patients who were treated by dietary measures alone, gained on the average 1070 grams per person per week for 54 weeks The average total weight gain was 58 kilograms, which was equivalent to 14 per cent of the average initial weight.

During the dietary periods, the consistently low levels of basal heat output rose from an average of 491 calories per hour to 535 calories per hour or 9 per cent In view of the average increase of body surface of 6 per cent which accompanied the weight gain the average basal metabolic rate changed only 2 per cent.

An increase of weight of 14 per cent is therefore accompanied by an increase in basal heat production of 9 per cent although this fact is obscured if heat production is expressed solely in terms of basal metabolic rate.

*Successful Orchidectomy in Addison's Disease* By CARL H GREENE and L. G ROWNTREE and (by invitation) WALTMAN WALTERS Rochester, Minn.

Our experience has shown that any surgical operation on a patient with untreated bilateral renal tuberculosis or Addison's disease usually serves to initiate a crisis of acute adrenal insufficiency with death within one to sixteen days A case is reported of a man of thirty-four, with frank Addison's disease, in whom a successful orchidectomy was performed in consequence of the preoperative and postoperative use of adrenal cortical hormone, prepared by the method of Swingle and Pfiffner The relation of this type of treatment to surgical practice in general is discussed.

*The Effect of Ingested Fat and of Acidosis upon the Hyperlipemia of Diabetes Mellitus* By ARTHUR C CURTIS and (by invitation) JOHN SHELDON, Ann Arbor, Mich

Three cases of diabetes mellitus with hyperlipemia were studied. The first case showed a decrease in the blood fat although large amounts of fat and a high caloric diet were ingested When glycosuria and acetoneuria were induced by removing insulin a slight increase in blood fat occurred The second case had a lipemia of 11.6 per cent. This rapidly fell to 0.7 per cent on a high fat and high caloric diet with insulin. The insulin was then slowly decreased and



finally omitted. No change in the blood fat was evident until acidosis developed. With acidosis the blood fat rose to 3.65 per cent. The third case had a lipemia of 14.16 per cent. This rapidly fell as the diabetes was controlled to 1.40 per cent although the patient was ingesting large amounts of fat. The subsequent removal of insulin was then accompanied by a gradually increasing glycosuria until it became as great as 206 grams in 24 hours. At this time no change in the  $\text{CO}_2$  combining power was present and little change in the blood fat had occurred. The acidosis subsequently increased until the  $\text{CO}_2$  combining power was 30 mgm per cent and the acetone bodies in the urine rose from 0.002 gram per 100 cc of urine to 0.323 gram per 100 cc of urine. Accompanying the mounting acidosis there was a steady increase in blood fat until at the height of acidosis it was 6.40 per cent. The addition of insulin alone to the previous regimen caused a fall in blood fat from 6.40 per cent to 0.95 per cent in 12 days.

#### *Conclusions*

- 1 Lipemia has been reproduced in patients having diabetes mellitus
- 2 The return of the lipemia is not directly related to ingested fat or to caloric intake
- 3 It occurs when the carbohydrate combustion is so diminished that acidosis is produced
- 4 The inability to oxidize fat causes its increase in the blood. By addition of insulin, carbohydrate is oxidized and lipemia disappears

#### *Influence of Hormones on a Glycogen-splitting Enzyme in Malignant Tissue*

By FREDERICK H. SCHARLES (by invitation) and WILLIAM T. SALTER, Boston, Mass.

In work directed toward the enzymic activity of cancer cells (summarized for this society in 1931) we found no production of lactic acid by mouse sarcoma (number 180) from mouse glycogen. Barr et al. had previously reported a similar result. The reasons for this finding are two: first, although tumor cells contain an enzyme which does split glycogen, lactic acid is not produced by this tumor amylase; secondly, the intracellular amylase will not effect extracellular glycogen.

This enzyme, which is extractable from tumor by water, differs from other tissue amylases. Its optimum temperature, in vitro, is  $48^\circ\text{C}$  rather than  $37^\circ\text{C}$ . The optimum pH for activity is 6.2. Glycogen is hydrolyzed, not into lactic acid, but into what are probably several compounds which yield a net reducing value of about one-third that of the inherent glucose. The glycogenolytic power of the enzyme under suitable conditions in vitro varies directly as the logarithm of its concentration. Of more fundamental import, however, is the fact that hormones can change the effective concentration of the enzyme in the tumor. Insulin causes an increase of 35 per cent and thyroxin of 150 per cent. Adrenalin and starvation cause no demonstrable change. It is not poisoned by cyanide nor iodo-acetate in vitro, nor does radiation with high voltage x-ray diminish its activity.

#### *The Consumption of Blood Sugar by Muscle in the Non-Diabetic and Diabetic State*

By WALLACE M. YATER and (by invitation) J. MARKOWITZ and RUSSELL F. CAHOON, Washington, D. C.

- 1 Heart muscle of a normal heart-lung perfusion consumes 4.0 mgm of glucose per gram per hour, as was previously shown by Knowlton and Starling and Cruikshank.

2 Heart muscle of a diabetic heart lung perfusion consumes less than one-fourth this quantity This figure corresponds to the requirement of resting skeletal muscle of liverless dogs

3 The decline in blood sugar following removal of the liver in dogs is the same in both the non-diabetic and the diabetic state.

4 The injection of 0.25 gram glucose intravenously per kilo per hour in liverless dogs suffices to maintain a normal level of blood sugar, as was previously shown by Mann and Magath

5 When liverless dogs are subjected to severe strychnine spasms the continuous injection of glucose at this rate is insufficient to maintain the blood sugar and it falls progressively to a hypoglycemic level in 5 hours

6 When diabetic liverless dogs are injected intravenously with glucose at the rate of 0.25 gram per kilo per hour after the blood sugar has declined to a non-diabetic level there is a slowly progressive rise of the blood sugar level It is estimated that the blood sugar would be maintained at an approximately normal level by the injection of 3/16 gram of glucose per kilo per hour

7 On the basis of these experiments and those quoted from the literature the hypothesis is propounded that in the diabetic state the glucose requirement of resting skeletal muscle is the same as in the non-diabetic state, but that in the diabetic state contracting muscle is unable to utilize the glucose molecule for purposes of obtaining the extra energy Apparently it is a function of insulin to elaborate the glucose molecule so that it is available for this purpose.

*The Manner of Removal of Protein from Normal Joints* By CHARLES L. SHORT and GRANVILLE A. BENNETT (by invitation) and WALTER BAUER, Boston Mass

Information concerning the manner in which proteins are removed from joints as well as the rate of removal is necessary for a better understanding of the physiology of normal joints, and the factors involved in the production and maintenance of joint effusions

These experiments were performed on the knee joints of normal dogs Various dilutions of egg albumin were injected into the knee joints and the time of its appearance in the blood stream was determined by precipitation tests done at frequent intervals using the serum of rabbits immunized against egg albumin It was definitely shown that protein is removed much more rapidly from passively exercised joints than from joints at rest In the case of the exercised joints, the protein could be detected in the serum as early as thirty minutes after the intra articular injection That there was but little tendency for the egg albumin to accumulate in the serum was evidenced by the fact that the amount contained in the serum did not increase markedly This finding was confirmed by following the egg albumin content of the serum in dogs receiving intravenously small injections of egg albumin at frequent intervals

In certain experiments the lymphatic vessels were tied off at their entrances into the larger vessels of the neck In such instances no egg albumin appeared in the blood stream three hours after the intra articular injection of egg albumin thereby demonstrating conclusively that protein is removed from joints only by way of the lymphatics

Similar experiments have been done in which horse serum and the albumin and globulin fractions of horse serum were injected into active and inactive joints instead of egg albumin

These experiments aid one in concluding that in cases of long standing joint effusions, the removal of proteins is definitely interfered with In such cases

one can state that there is definite interference with lymphatic drainage. This information suggests that a test might be devised which would enable one to state more definitely what therapeutic procedures are indicated in the treatment of a given case with a joint effusion.

*The Treatment of Cardiovascular Syphilis* By J E MOORE and (by invitation) J H DANGLADE and J E REISINGER, Baltimore, Md

This is a preliminary report of the outcome in 165 patients (112 with aortic regurgitation and 53 with aneurysm of the aorta) who received the same type of general medical care, but varying amounts of anti-syphilitic treatment. The scheme of treatment emphasized is based on the avoidance of therapeutic shock (the Herxheimer reaction), of the therapeutic paradox, and of drug, particularly arsenical, reactions. It is shown that by adequate treatment, as compared with little or none, symptoms may be alleviated, the mortality rate is reduced (during an observation period of 5-10 years) from 92 to 25 per cent, and the average duration of life after the onset of symptoms is increased from an approximate 24 months to 75 months or longer.

*General Edema of Indeterminate Origin* By NORMAN M KEITH, Rochester, Minn

Five cases of general edema, indeterminate in origin, were seen between June, 1924, and July, 1925. The ages of the patients varied from nine to sixty-nine years. Edema had been present in one case for two years, and in the other four cases from two to four months. One patient gave a history of syphilis, one, a history of arrested chronic pulmonary tuberculosis, and three patients of previous good health. There was no distinct cardiovascular abnormalities nor any anemia. Daily urinalysis sometimes disclosed nothing abnormal, whereas, at other times, casts and traces of albumin were present. In two cases, the values for serum protein and blood cholesterol only were determined, in one case these values were normal, in the other, the value for serum protein was reduced to 4.6 mgm. in each 100 cc. and that for blood cholesterol was increased to 325 mgm. in each 100 cc. Treatment consisted of a diet low in salt and water, and administration of certain diuretics. In four cases the results were very satisfactory, in the fifth case, a large amount of fluid was eliminated through punctures in the skin of the legs. The ultimate prognosis in all cases was good. One patient, however, died five and a half years later with apparent cardiac failure and symptoms suggesting coronary occlusion. The most likely causative factors of the edema would seem to be some general toxic or nutritional disturbance.

*Non-specific Versus Specific Serum Treatment in Lobar Pneumonia* By M FINLAND and W D SUTLIFF (by invitation) and R. N NYE, Boston, Mass

A direct comparison between the response to the therapeutic administration of horse serum globulin solutions containing no specific antibodies and horse serum globulin solutions containing a high concentration of specific antibodies might be expected to provide an adequate control for the evaluation of the effect of specific serum and incidentally to throw some light on the nature and extent of the therapeutic response to non-specific therapy.

A concentrated solution of horse serum globulins was prepared from anti-meningococcic serum. Some patients were treated non-specifically with this serum and 20 to 60 hours later, were given equivalent amounts of antipneumococcic serum. Others received only non-specific therapy. The latter cases were compared with cases given specific therapy alone.

It was observed that non specific therapy of the sort described had a temporary antipyretic effect, produced a temporary sense of well being in the patient, occasionally resulted in a temporary sterilization of the blood stream, but had no perceptible influence in preventing extension of the lesion. Specific serum therapy had a permanent antipyretic effect, produced a permanent amelioration of subjective complaints, regularly sterilized the blood stream and prevented extension of the pneumonic process.

It is concluded that the curative effects of Type I antipneumococcic serum in the treatment of Type I lobar pneumonia are due to its content of specific antibodies rather than to its action as a foreign protein.

*Immune Reactions in Diabetic Patients* By HOBART A. REIMANN and (by invitation) JOHANNES K. MOEN, Minneapolis, Minn.

Several theories account for the increased susceptibility of diabetic patients to infection, (a) increased sugar in the tissue favors the growth of invading organisms, (b) diminished bactericidal capacity of the blood, (c) reduction of the opsonic index, (d) lack of tissue resistance.

Because of the correlation found between the suppressed formation of agglutinins and the tendency to secondary infections in leukemia and kala-azar, similar experiments were performed in diabetic patients. Four severe, 8 moderately severe and 4 controlled diabetic patients together with 10 normal controls were vaccinated in the usual manner with typhoid, paratyphoid A and B vaccine. Blood agglutination tests were made 10 days after the first injection and thereafter at 10 day intervals for 2 months.

The agglutinins appeared as usual in the normal individuals in high titer, occasionally as high as 20 000. The agglutinin titer was lower in most of the diabetics. The response was subnormal in the controlled diabetics, and distinctly low in the moderate cases. In 2 patients vaccinated during acidosis and glycosuria scarcely any agglutinins appeared.

*Conclusion* The ability of diabetic patients to produce agglutinins following antityphoid vaccination is diminished. This probably indicates another factor to account for the increased susceptibility of diabetic patients to infection.

*Study of Acute Respiratory Disease in Spitzbergen* By W. G. SMILLIE and (by invitation) J. H. PAUL and H. L. FREESE, Boston, Mass.

As a contrast to our year's study of "colds" in the Virgin Islands, West Indies, we selected Spitzbergen. This is a small isolated community, subjected to rigorous climatic conditions. The study began September 1, 1930. The ice closed down in October and no ships came in until June, so that isolation was complete. Almost no respiratory disease occurred in the population (500) during the winter. A severe epidemic struck with the arrival of the first ship in June. Bacteriological studies of nasopharyngeal flora of the people were made throughout the year, as well as observations of environmental conditions, including temperature, precipitation, relative humidity, sunlight, wind velocity and air ionization. Our results show that the "colds" in Spitzbergen were contagious, spread by direct contact, with an incubation period of about 36 hours. Environmental factors played no part in the incidence. None of the familiar organisms of the nasopharyngeal flora could be incriminated as etiological agents.

*The Anginal Syndrome Induced by Gradual General Anoxemia* By MARCUS A. ROTHSCHILD and (by invitation) MILTON KISSIN, New York, N. Y.

With individuals subject to attacks of chest pain, in whom there is no evidence by physical or electrocardiographic examination of myocardial (coronary)

disease, it is often difficult to be certain of the origin of the pain. We wished to distinguish in these cases between those with pain due to impaired coronary circulation, and those in whom the pain arose otherwise. It occurred to us that if one were to produce a general anoxemia, and therefore a local cardiac anoxemia, there might appear differentiating responses in these two groups.

By means of rebreathing, we were able to produce a state of general anoxemia in human subjects. The carbon dioxide was absorbed. It usually took about ten minutes for the oxygen to become so low that the patient became uncomfortable.

Twenty-six patients were subjected to the test. Fourteen patients were used as controls. The controls consisted of four patients with normal hearts. The remaining ten cases were patients with chronic valvular disease, paroxysmal auricular fibrillation, rheumatic fever, spondylitis, gallbladder disease, and cardiospasm. None of these patients developed pain. Twelve were patients with clear-cut histories of attacks of precordial pain brought on by exertion, excitement, eating, or exposure to cold. Nine developed pain during the rebreathing test. Seven of these had no physical or electrocardiographic signs of myocardial disease. The pain appeared when the oxygen fell to about 9 to 10 per cent. This ordinarily took about 8 to 10 minutes. Subjects were advised to raise their hands when they felt uncomfortable, and the experiment was stopped.

It is furthermore interesting to observe that two other patients with clinical angina and intraventricular block developed pain and additional electrocardiographic changes during the anoxemia. The changes found were depression of the R-T segments in Leads II and III, and in another instance in Leads I and III. Unfortunately before further tests could be made, the second patient died, but the patient with changes in II and III was observed further. We were unable to reproduce the electrocardiographic changes with (1) oxygen and carbon dioxide inhalations, (2) intravenous atropin, (3) adrenalin, (4) pitressin, and (5) amyl nitrate.

There were three patients with clinical coronary artery disease, one with definite electrocardiographic changes, and two with no electrocardiographic changes, that responded negatively to the test.

We feel, therefore, that it is possible to reproduce the pain of angina pectoris in susceptible subjects by inducing general anoxemia. We assume in the explanation that the coronary circulation, normally adequate, becomes inadequate during anoxemia. We are not prepared to state that anoxemia per se is responsible for the pain that appears during the experiment. It is possible that the explanation of angina as being due to a cardiac ischemia combined with a piling up of Lewis's P-factor explains the pain that appears during rebreathing. We feel that our work, although not conclusive, tends however to support the anoxemic theory of angina pectoris. This test promises to be of some value in differentiating the causation of chest pain.

*The Cause of Dyspnea on Mild Exertion in Persons with Cardiac Disease* By T. R. HARRISON and (by invitation) W. G. HARRISON, JR., and J. A. CALHOUN, Nashville, Tenn.

Observations have been made regarding the increase in ventilation produced by mild exercise in persons with cardiac disease. Previous studies have shown that such exertion is not associated with changes in the composition of either the arterial blood or the blood returning from the brain. Hence the increase in ventilation is not due to changes in cerebral blood flow.

It has been found that muscular movement causes a reflex increase in respiration. The evidence is as follows:

1 Moving the hands causes increased ventilation even though the circulation to and from the arm is occluded by an inflated blood pressure cuff around the arms.

2 Passive movements of a dog's leg cause increase in ventilation. If the spinal cord is cut in the mid-dorsal region moving the hind leg does not, but moving the fore leg does cause increase in ventilation.

3 If the hind leg of a dog be amputated except for the sciatic nerve and femoral vessels, movements of the leg cause increase in ventilation whether or not the vessels are obstructed. Cutting the nerve abolishes the effect.

Patients with cardiac disease have a greater ventilation for a given exercise than do normal subjects. They likewise have a greater and more sustained rise in venous pressure. Experiments on dogs indicate that the latter phenomenon may be a cause of the former because (a) rapid intravenous injection of fluid causes increase in ventilation when the vagus nerves are intact but not when they are cut, (b) increasing the venous pressure by inflating a balloon in the right auricle also increases, and deflation of the balloon decreases the ventilation. No such effects are obtained by the same procedures after bilateral vagotomy.

It is concluded that the dyspnea produced by mild exertion in persons with cardiac disease is due to reflex respiratory stimulation from the moving muscles and from the heart.

*Intra-Capillary Anastomoses* By IRVING WRIGHT (introduced by H. O. Rosenthal), New York, N. Y.

We have observed in numerous patients small anastomosing vessels which connect different segments of an individual capillary loop. Some of these connect the arterial limb with the venous limb, others two portions of the venous limb, other patterns also have been seen. These vessels are smaller in diameter than the capillaries with which they are associated.

Two major considerations will be discussed.

1 The blood may cease to flow through these anastomoses while it still continues to flow through the capillary, passing the openings of the anastomoses. If this is due to an independent contraction of these minute anastomoses it would be valuable evidence in favor of the presence of an independent neuromuscular mechanism, probably the Rouget cells. Our observations lead us to believe that such is the case.

2 We have considered the development of these vessels. More than one anastomosis is rarely seen in the nail fold capillaries of a single patient, and it is impossible to see them in the capillaries elsewhere in the body. We have been able to see them more frequently since we have adapted the ultra-pak to capillary study.

These are probably residual vessels from the archiform capillaries of early infancy. We have confined these studies to mature adults in order to avoid confusion with the normal network seen in early infancy and sometimes present in childhood especially in cretins.

Whether human capillaries bud out and produce such anastomoses we cannot say at present but studies are being made of this possibility.

Finally, these are not the arteriolar venule anastomoses described by Heimbürger, Lewis and others.

*The Clinical Value of Electrocardiograms Taken in an Anteroposterior Plane at Right Angles to the Frontal Plane of the Classical Electrocardiogram* By EDWARD F. BLAND, SYLVESTER MCGINN and HOWARD B. SPRAGUE (by invitation) and PAUL D. WHITE, Boston, Mass

*On the Relationship between Cardiac Size and Basal Cardiac Work in Common Clinical Conditions* By ISAAC STARR, JR., and (by invitation) LEON H. COLLINS, JR., and FRANCIS C. WOOD, Philadelphia, Pa

Estimations of cardiac output (method of Starr and Gamble presented before this society in 1928) and of blood pressure permit calculations of cardiac work by Starling's formula. Duplicate determinations were made in the postabsorptive state on fifty cases, viz. normals, threatened congestive failure, angina pectoris, hypertension, anemia, thyrotoxicosis and functional heart disease.

The relationship between cardiac size and work per beat has clinical significance dividing the cases threatened with congestive failure from the other cases. The latter arrange themselves along a straight line, the great majority deviating from it less than could be accounted for by the large errors inherent in the estimations, the former are far distant. Therefore in normal conditions the work of the heart is a function of its size, an extension of Starling's "Law of the Heart" to clinical conditions. In failure large hearts do but little work.

The relationship between heart size and heart work per beat was especially striking in 17 cases of hypertension. Those with hearts of normal size, by reducing cardiac output, maintained their hypertension without greater expenditure of cardiac work than normal persons. The cases with large hearts were performing increased work. Considering increased cardiac work as cause of hypertrophy in the latter group, its absence will explain the absence of hypertrophy in the former.

*Comparison of the Way in Which Normal and Diseased Kidneys Excrete Waste Products* By F. H. LASHMET (by invitation) and L. H. NEWBURGH, Ann Arbor, Mich

Under conditions which markedly restrict the fluid, but not the solid intake of the body, normal kidneys are able to excrete concentrated urine of high specific gravity. Under identical conditions, diseased kidneys excrete dilute urine of low specific gravity. This difference was at first supposed to be due to the inability of diseased kidneys to excrete the normal amount of waste products per unit of time.

If, however, the total amount of waste products and the volume of urine excreted per unit of time are determined, diseased kidneys eliminate solids at a high rate, provided sufficient water is available to permit the required dilution. The increased volume of urine is obtained at the expense of body water. The extent of the increase in urine volume is inversely proportional to the maximum specific gravity which the diseased kidneys can attain under the specified conditions.

The presence of edema changes somewhat the above findings. During the period of water restriction, patients with edema excrete urine of the expected low specific gravity, but of small volume and low total solids. If they are then allowed to drink water freely, they will excrete not only the normal amount of waste products in an increased volume, but also those retained from the period during which water was restricted.

These findings reveal the importance of adequate fluid intake in all cases of nephritis, including even those with edema. If there is to be no retention of

waste products, a large volume of urine must be assured by an adequate water intake.

*The Heart Rate in "Frizzle" Fowls its Bearing on the Human "Goiter" Heart*  
By ERNST P. BOAS, New York, and (by invitation) WALTER LANDAUER  
Storrs, Conn

It is still unknown in how far the increased metabolism, as such, or in how far altered thyroid secretion causes the cardiac disturbances of Graves' disease. The Frizzle fowl is a variety of chicken with peculiar upward turning feathers and a very scanty plumage. The lack of feathers causes an excess loss of body heat, and a compensatory increase in basal metabolism. These chickens may live for a number of years and so offer a unique opportunity of studying the effect of a permanently high metabolism on the heart.

We have studied the heart rate of 22 Frizzle fowls and of 27 normal chickens of the same approximate age and weight. In some chickens the heart rate was counted for periods of several hours by means of the cardiograph. The usual electrodes applied to the skin of the breast were employed. Electrocardiograms were taken with needle electrodes. All readings were obtained while the chicken lay quietly on its back without struggling.

The heart rate of normal chickens weighing about 1.5 kilograms and about one year old ranges from 180 to 330 a minute. This is lower than rates reported in the literature which range around 350, and which are undoubtedly too high because the conditions of the experiments were far from basal. The heart rate of Frizzle chickens of the same weight and age ranges from 260 to 440. The average minimum rate per minute of the normal chickens was 263, of the Frizzle fowl 334.

It seems that in the Frizzle chicken the increased basal metabolism alone conditions a permanently increased heart rate. In human subjects with Graves' disease there must be a similar effect. The cardiac hypertrophy which has been observed in the Frizzle chicken is apparently related to this increased work of the heart. A similar mechanism may account, in part at least, for the cardiac enlargement that occurs in Graves' disease.

*Cataphoretic Potential of Streptococci as Isolated in Studies on Arthritis* By  
EDWARD C. ROSENOW, Rochester, Minn.

The paper consists of a report on the cataphoretic potential of streptococci when isolated, and after long cultivation obtained from various atriæ of infection from the stool and from the blood in various forms of arthritis and allied conditions, and the effect of the patient's serum in lowering the cataphoretic potential of the streptococcus.

*Renal Function During the Dissipation of Cardiac Edema* By GEORGE HERRMANN and (by invitation) E. H. SCHWAB and W. W. BONDURANT, Galveston, Texas

Studies of the creatinine, urea, sodium chloride and the  $\text{CO}_2$  in the blood and the urine during the mobilization and excretion of fluid under the influence of xanthine and mercurial diuretics and digitalization has yielded some interesting data on the mechanism of the action of the latter procedures. Applying Rehberg's formulae it is apparent that the diuresis from xanthine and digitalis bodies in so far as the kidney is concerned in patients with congestive heart failure is accompanied by great increase in filtration with but little decrease in reabsorption while with the mercurials the diuresis was accomplished primarily by a de-



crease in the reabsorption phenomenon. The blood  $\text{CO}_2$  dropped as the base was lost through the kidney. The sodium chloride maintained about a normal level in the blood while the urinary output increased tremendously. The blood urea level rose conspicuously and dropped promptly back to just above the normal while the urinary excretion increased considerably during the height of the diuresis and dropped off sharply to below the resting level in ten hours.

*Observations on Heart Sounds with Particular Reference to Gallop Rhythm and Sounds of Auricular Origin* By A. G. MACLEOD (by invitation) and FRANK N. WILSON, Ann Arbor, Mich.

Simultaneous phonocardiograms and electrocardiograms were taken in a series of patients with normal and pathologic hearts. A study of these records permits the following tentative conclusions:

1. In certain cases of heart block the R-T interval may be nearly twice as long as the interval between the first and second sounds.

2. Auricular systole may produce sounds as loud as, or louder than, the first or second heart sound. It often produces a double sound, the second element of which follows the P wave by a considerable interval. There are several factors which determine the intensity of the auricular sounds. Audible auricular sounds may occur in midsystole.

3. In bundle branch block associated with gallop rhythm the extra sound is presystolic and is not caused by asynchronous contraction of the ventricles.

4. In gallop rhythm the extra sound may be protodiastolic, presystolic or systolic. Presystolic gallop rhythm is by far the most common variety. In this type the extra sound seems to be of auricular origin. One interesting case was observed in which both presystolic and protodiastolic gallop rhythm were present at the same time. At ordinary rates one very loud mid-diastolic sound was heard, at slower rates this was separated into its two components.

*The Electrocardiogram in Coronary Thrombosis* By PAUL S. BARKER and (by invitation) L. L. KLOSTERMYER and A. G. MACLEOD, Ann Arbor, Mich.

The electrocardiogram is useful in the diagnosis of coronary thrombosis, particularly when a series of curves is possible.

An important feature of the abnormal T-deflections is their progressive change in form. This is accompanied by QRS changes which have not been fully described. The curves fall into two groups.

The first group shows small complexes in lead I with a conspicuous and broad Q-deflection. In leads II and III the first deflection of QRS is upward, followed by an S-wave often of large amplitude. These QRS changes are associated with a sharply inverted T-wave in lead I in the late stages of coronary thrombosis, and with an upright T partially fused with QRS in the early stages.

The second group shows prominent Q-waves in leads II and III and T-wave changes of the opposite type. Corresponding to these changes there are characteristic changes in precordial leads which are of value in understanding how these phenomena are produced.

Changes in heart rhythm, particularly the occurrence of partial or complete block, paroxysmal ventricular tachycardia, and the sudden development of intraventricular block, or of very small complexes are also common in coronary thrombosis and are of diagnostic value.

*Toxic Goitre and its Relation to Protein Digestion (Pancreatic)* By JOHN STAIGE DAVIS, JR. and JOHN A. KILLIAN (introduced by Carl Binger), New York, N. Y.

The concentration of tyrosine and tyramine has been estimated in the blood and urine of a series of patients suffering from various types of toxic goitre. These results have been compared with those of a group of patients exhibiting nontoxic thyroid disease, and of normal subjects. When studied in relation to the basal metabolic rate, pulse rate and weight, the tyrosine and tyramine concentrations appear to have a definite relationship to the severity of the clinical manifestations.

Observations derived from animal experiments are presented which show that changes in the histopathology of the thyroid gland occur following ligation of the pancreatic ducts. These changes strongly resemble colloid goitre in man and are believed to be due to the lack of tyrosine production.

The general theoretical conclusion is reached that the absorption of the split products of protein digestion in the small intestine is related to the phenomena of thyroid disease. More specifically, that an excess in the concentrations of tyramine and tyrosine in the blood may be responsible for the manifestations of thyrotoxicosis, while the absence of these substances may result in colloid degeneration of the gland.

*The Redistribution of Vital Capacity After Paralysis of the Hemidiaphragm*  
By JOSEPH W. GALE (by invitation) and WILLIAM S. MIDDLETON, Madison, Wis.

For a period of time after phrenic block or evulsion the vital capacity is perceptibly reduced as a rule. In certain instances after some time has elapsed the vital capacity returns to the preoperative level and may even exceed the same. Two circumstances explain this change, namely redistribution of the aerating space and decrease in the toxemia of the underlying process. The first of these factors can be evaluated and by a standard technique the area of the two hemithoraces has been determined in a series of cases before and after phrenic paralysis. The several possibilities as revealed by this method are discussed.

*The Respiratory Metabolism of Acid Fast Bacteria as Influenced by Foodstuffs, Narcotics and Methylene Blue* By R. O. LOEBEL, E. SHORR and H. B. RICHARDSON, New York, N. Y.

Utilization of foodstuffs by bacteria of the acid fast group was described in an earlier paper and the relation of the findings to the survival of the bacteria within the body was discussed. The substances which were associated with an increase in respiration included glucose, lactic acid, glycerol, soaps and lecithin. Reasons were given for supposing that this increase denoted an actual consumption of the foodstuff in question rather than a non specific stimulus of oxidation. Additional evidence has now been obtained on this point by the study of the respiratory quotient, as affected by two groups of substances. The first group consisted of foodstuffs, the second of substances which are known to affect oxidation without necessarily undergoing oxidation. The latter group included the narcotics KCN and ethyl urethane, and in addition methylene blue.

The organism used for the study was *B. phlei*, the timothy bacillus. The bacteria, after remaining for several days on a non nutrient medium, were transferred to fresh fluid with or without the substance to be tested. The

R. Q without nutriment was 0.75 to 0.81, with glucose 0.96 to 1.01, with sodium lactate 0.98 to 0.99, with sodium stearate and palmitate 0.74 and 0.77. With one exception the quotients observed corresponded to the theoretical value of the substance in question. This exception was sodium oleate which yielded quotients of 0.97 and 0.91, thereby resembling the second group of substances.

Both KCN and ethyl urethane caused, in addition to the expected inhibition at high concentrations, an increase in respiration in appropriate dilutions. This occurred both in non-nutrient media and in 0.2 per cent glucose, but was not apparent in lipoids. The respiratory quotients were 0.71 to 0.86 in the absence both of food and narcotic, 0.90 to 1.07 in the presence of KCN M/500 to M/5000, and 0.97 to 1.08 in 0.3 per cent ethyl urethane. Methylene blue had an action like the dilute narcotic but less marked. Since KCN can hardly be supposed to undergo oxidation, and since all three substances have the same effect on oxidation although they have nothing in common chemically, it is to be inferred that they act by increasing the oxidation of carbohydrate or other substance of high respiratory quotient.

In summary, the evidence from the respiratory quotient indicates that the foodstuffs tested are actually oxidized, in contrast to dilute KCN, dilute ethyl urethane, and 0.05 per cent methylene blue which stimulate respiration and cause a rise in the respiratory quotient, due probably to an increase in the oxidation of carbohydrate.

*The Hemoglobin and its Variations in the Blood of Normal and Anemic Persons* By WM P MURPHY and (by invitation) I M HOWARD, Boston, Mass.

Studies of the hemoglobin in the blood and in crystalline form have been made. Its minimal molecular weight and percentage composition of iron have been calculated.

These are discussed together with certain relations of the hemoglobin in the blood of normal and anemic persons, particularly as influenced by treatment.

*The Hypothalamus and Blood Pressure Regulation* By LOUIS LEITER and (by invitation) ROY R GRINKER, Chicago, Ill.

Various investigators have assumed the existence of a superior vasomotor center in the hypothalamus. The experimental and clinical evidence for this hypothesis is meager, but the apparent demonstration of other vegetative centers in the diencephalon and the possible rôle of the hypothalamus in clinical hypertension seemed to justify another direct attack upon the problem.

In a large series of cats, the hypothalamic region was stimulated with the faradic current after exposure of the base of the brain from above or through the roof of the mouth. The site of the electrode was controlled histologically. The type and degree of anesthesia and the strength of stimulus were varied. The blood pressure and respiration were recorded and muscular movements carefully observed.

The results indicated that the blood pressure might rise, fall or remain unchanged during stimulation of the hypothalamus. Almost invariably, however, increases in blood pressure were associated with convulsive movements. The two phenomena ran roughly parallel in degree, regardless of the area stimulated. After the diminution or suppression of muscular activity by curare, no rise in blood pressure occurred upon excitation of the hypothalamus unless a very strong stimulus was used, in which event, the same result could be obtained from other parts of the brain.

These experiments would seem to rule out the presence of a hypothalamic center, in the cat, for the regulation of blood pressure.

*The Control and Complete Remission of Polycythemia Vera Following the Prolonged Administration of Phenylhydrazine Hydrochloride* By H Z GIFFIN and (by invitation) E. V ALLEN, ROCHESTER, MINN.

A group of thirty seven patients with polycythemia vera, who were observed or given initial treatment during the years 1925, 1926, and 1927, has been reviewed in order to obtain information concerning the remote effects of treatment. Twenty five of the patients received an initial course of phenylhydrazine hydrochloride at home. Brief abstracts of the records of seven patients, of whom adequate data were obtained and who had had an adequate amount of the drug, are presented. The records of these seven patients illustrate the excellent control which may be obtained, after the initial course of treatment, by means of the administration of small doses of phenylhydrazine hydrochloride. The condition of three of the seven patients, after prolonged treatment, underwent complete remission, varying from five months to one and a half years.

The toxicity of phenylhydrazine hydrochloride and the development of tolerance to the drug are considered. After the initial course of treatment it seems best not to allow erythrocytosis to recur but to keep the erythrocyte count and the blood volume under control with a sufficient amount of phenylhydrazine hydrochloride each week, usually from 0.1 to 0.3 gram.

*A Rapid Method for Determining Magnesium in Blood and Urine* By ARTHUR D HIRSCHFELDER and (by invitation) EARL R. SERLES, Minneapolis, Minn.

Kolthoff (1926) showed that traces of magnesium give a pink color with two yellow acridine sulpho dyes (Titan Yellow and Clayton Yellow) and that by comparison with a standard magnesium solution this reaction can be used for colorimetric determination of magnesium. Presence of calcium intensifies the color. We have used this method for determination of magnesium in blood plasma, from which the calcium has been removed by a modified Kramer-Tisdall method. The determination is very rapid, and simple. The results check closely with those obtained by methods of Denis and Briggs, and when known amounts of magnesium are added to plasma the figures obtained check closely. By using a microcolorimeter magnesium can be determined accurately in 0.1 cc. plasma. In urine, uranium acetate must be added to remove phosphates and blood pigment as well as oxalating out the calcium after which the magnesium can be determined accurately.

Purgative doses of magnesium sulphate given to normal men, dogs or rabbits does not raise blood magnesium significantly, but in nephrectomized dogs they cause blood magnesium to rise to 18 to 20 mgm. per 100 cc. plasma, and the dogs promptly go into coma. Rabbits rendered nephrotic by  $HgCl_2$  react similarly and can be aroused by intravenous  $CaCl_2$ .  $Na_2SO_4$  does not produce coma in nephrotic rabbits. It, therefore, seems probable that coma simulating uremic coma can be caused by Epsom salt purgation in patients with badly diseased renal tubules.

*Study of the Gastric Secretion in Hyperthyroidism Before and After Operation* By W R. BERRYHILL and H A. WILLIAMS (introduced by M A Blankenhorn), Cleveland, Ohio

Gastric analyses following histamine were done on fifty patients with hyperthyroidism before and after operation. Before operation thirty four had an

achlorhydria, thirteen hypoacidity and three a normal acidity After the hyperthyroidism was relieved by operation, twenty-six patients with previous achlorhydria were carefully followed, nineteen of whom had a return of normal acidity The thirteen cases with hypoacidity returned to normal This work shows a high incidence of achlorhydria in hyperthyroidism, but also a proportionately large return to normal acidity following the relief of the hyperthyroidism

*The Degradation of Mycobacteria into Non-acid-fast Forms* By F R. MILLER (introduced by Gerald S Shibley), Cleveland, Ohio

Six strains representing four types of mycobacteria were grown in contact with the Berkefeld filtrates from a non-acid-fast chromogenic strain of H37 human tubercle bacilli These six strains included three strains of human tubercle bacilli, one strain of bovine tubercle bacilli, one strain of smegma bacilli and one strain of timothy grass bacilli From this treatment of acid-fast organisms seventeen non-acid-fast growths were obtained At first these latter were all quite similar, being made up of non-acid-fast coccoid and rod forms and showing small gray-white colonies in twenty-four to forty-eight hours on agar Four of these non-acid-fast growths, so far, have again developed acid-fast forms and colony formations similar to the parent strains from which they originated

The filtrates of the chromogenic H37 organisms were sterile when plated for long periods of time on various media The growth of non-acid-fast forms also occurred when acid-fast organisms were cultured in contact with filtrates which had been autoclaved

A further study of acid-fast organisms by microcultures of single cells in contact with both autoclaved and unautoclaved Berkefeld filtrates of chromogenic H37 organisms is resulting in similar non-acid-fast growths

*Distribution of the Blood Entering the Coronary Arteries* By A R MORITZ, C L HUDSON and E S ORGAIN (introduced by Elliott C Cutler), Cleveland, Ohio

In order to study the extent and distribution of the blood supply entering the coronary arteries experiments were made by injecting an opaque substance into the coronary arteries at a pressure of 220 mm Hg with the heart in situ The coronary arteries were cannulated by making a small slit in the wall of the aorta It was found that the substance injected into the coronary arteries filled the vasa vasorum of the aorta down to the level of the diaphragm, that the pericardium obtained a considerable proportion of its blood supply from the coronary arteries and that the diaphragm obtained a fair portion of its blood supply from the coronary arteries These vessels formed a rich anastomotic network over the organs mentioned It is felt that this distribution of blood, particularly in the wall of the aorta and in the pericardium, is of significance, particularly in luetic aortitis and other diseases of the aorta

PROCEEDINGS OF THE FOURTH ANNUAL MEETING OF  
THE CENTRAL SOCIETY FOR CLINICAL RESEARCH  
HELD IN CHICAGO, NOVEMBER 20, 1931

*The Influence of Work and of Thyroxine on the Metabolism of a Dog* By  
WALTER M. BOOTHBY, M.D. Rochester, Minn.

The effect of eleven intravenous injections of 10 mgm of thyroxine each on the metabolism of a dog over a period of nine months was studied. The experiment—to be reported in full by Boothby, Buckley and Wilhelmj (J. Physiol.)—was divided into three periods. The first period lasting eleven days, was without work and followed at least a year of confined cage life. In the second period of 115 days the dog was exercised on a treadmill. In the third period of sixty four days the exercise was again omitted. Throughout the experiment the dog was on a constant weighed diet containing 2.93 grams nitrogen and 480 calories each day. The nitrogen in the urine averaged 2.65 grams and in the feces and hair shed into the cage 0.24 gram daily, totaling 2.89 grams daily. Within the limits of experimental error average nitrogen equilibrium was maintained in all three periods. Immediately following the injection of thyroxine there is a temporary negative nitrogen balance followed by a compensatory positive balance after which equilibrium is attained. During period (1) the average respiratory quotient for all days, except the first four after each injection of thyroxine, averaged 0.82, for period (2), 0.81 and for period (3), 0.83. The dog lost weight in period (2) on account of the exercise, as he was in nitrogen equilibrium the loss of weight must have been due to loss of fat, the heat value of the fat corresponding to the loss in weight was calculated to be equivalent to the energy of the extra work, it was also calculated that this amount of fat would lower the average respiratory quotient from 0.82 to 0.81 as found experimentally. Similarly, in period (3) the dog gained in weight due to deposition of fat as nitrogen equilibrium existed the retention of this amount of fat would raise the average respiratory quotient from 0.82 to 0.83, as found experimentally. During the first four days after injection of thyroxine the average respiratory quotient was decreased to an average of 0.77, 0.75 and 0.76 in the three periods respectively, this decrease was greater than could be accounted for by the amount of fat needed to meet the increased energy requirements. The increased excretion of nitrogen immediately following administration of thyroxine was largely due to increased elimination of urea and to a slight extent to the elimination of creatine, the quantities of the other nitrogenous partition products did not vary significantly, the few days of compensating nitrogen retention was accounted for by a decrease in elimination of urea. The decay curve of thyroxine is more irregular in dogs than in human beings, but within the limits of experimental error it was consistent with the exponential form found in this laboratory for human beings. Following exercise on the treadmill the decay curve during period (2) decreased less rapidly than in the preliminary control period and this effect continued throughout the third period again without exercise. Under the conditions of this experiment the total calorigenic action of 10 mgm thyroxine averaged 220 calories in period (1) without exercise, 449 calories in period (2) with exercise and 487 calories in period (3) without exercise.

*Experimental Exophthalmos and its Relation to the Pathogenesis of Exophthalmic Goiter* By HARRY B FRIEDGOOD, M D (by invitation) and ALLEN M BOYDEN (by invitation), Ann Arbor, Mich (introduced by Cyrus C Sturgis, M D )

Faradic stimulation of the right superior cervical sympathetic ganglion in three dogs resulted in unmistakable unilateral exophthalmos, dilatation of the pupil and slight lacrimation, but both palpebral fissures widened perceptibly, especially on the right. Resection of this ganglion induced rapid enophthalmos and myosis. These changes were definitely demonstrated by a series of motion pictures of the experiment.

Exophthalmos, lid-lag and "lid-spasm" are infrequently seen in conditions other than exophthalmic goiter, for example, in essential hypertension and the "functional" psychoneuroses. These eye signs are not diagnostic of Graves' syndrome, but represent objective phenomena indicative of the state of tonicity in the sympathetic nervous system.

Increased nervous tension, secondary to any cause whatsoever, may be responsible for a markedly elevated basal metabolic rate. The action of epinephrine, an activator of the sympathetic nervous system, is also characterized by a definite rise in the basal metabolic rate. These data suggest that the sympathetic nervous system may be at least partially responsible for the elevated oxygen consumption in exophthalmic goiter.

Tachycardia, tremor, abnormal sweating, emotional and vasomotor instability, exophthalmos and associated eye signs are among the cardinal signs of sympathetic nervous system hyperactivity. The predominance of these nervous disturbances and the intensity with which they develop in Graves' syndrome suggest that the sympathetic nervous system plays a paramount rôle in its pathogenesis.

*Metabolic Studies During Morphine Withdrawal from a Human Addict* By DWIGHT C ENSIGN (by invitation) and FRANK J SLADEN, Detroit, Mich

Clinical studies emphasize the psychic factor in explanation of the symptoms following withdrawal of morphine. Studies upon animals, in which typical addiction may be produced, relate these symptoms to certain metabolic changes especially affecting carbohydrates and water. Directly opposite results have been obtained by different workers. Studies upon human subjects have resulted in similarly varying results. The authors' opportunity for study included a prolonged period of observation of a patient with a minimal psychic factor. The plan of treatment, precluding knowledge of reduction, was successful in avoiding the "before-the-next-dose" abstinence reaction. We report findings (1) before withdrawal, (2) during reduction, and (3) during the period of major symptoms of abstinence. Correlation of symptoms of withdrawal and findings

*A Clinical Study of the Cardiodynamics of Mitral Insufficiency and of Mitral Stenosis* By HAROLD FEIL, M D, Cleveland, Ohio

A brief discussion of the cardiodynamics in experimental acute mitral insufficiency and in acute mitral stenosis is presented. In a clinical study of fifteen cases of mitral insufficiency without signs of active infection or of failure the duration of the chief phases of systole and of total systole was normal. Twenty-one patients with clinical evidence of mitral stenosis were studied. Of twelve patients with normal mechanism eleven showed no deviation from the average duration of systole and of its chief phases, one patient with evidence of early cardiac failure had abbreviation of total systole and of ejection. Nine pa-

tients with auricular fibrillation all showed shortening of the ejection phase and of total systole. Mitral insufficiency and mitral stenosis cause no change in the duration of systole and of its chief phases unless failure or auricular fibrillation are present when shortening of total systole and of ejection occur.

These findings are in agreement with findings in these experimental lesions and it may be assumed that the compensatory processes in animals probably operate identically in man.

*Changes in the Electrocardiogram in the Course of Pericardial Effusion with Paracentesis and Pericardiectomy* By JOHN HARVEY, M D, and JOHN W SCOTT, M D, Lexington, Ky

In 1929 Scott, Feil and Katz reported the occurrence of the plateau type T wave characteristic of recent coronary occlusion in, (1) aneurysm ruptured into the pericardium and (2) purulent pericardial effusion. Since then considerable interest has been shown in the occurrence of such T waves in conditions other than coronary occlusion. Clinically it has been observed in pneumonia. In the experimental animal this type of T wave has been observed after coronary ligation, after injection of toxic material into the muscle of the ventricle, after a toxic dose of digitalis, after the injection of fluid into the pericardium and during induced general anoxemia.

Opportunity has been afforded to observe a case of pneumococcal pericarditis with massive effusion in which the plateau type T wave was present. Daily electrocardiograms taken in the course of the illness extending over seventeen days, during which paracentesis and later pericardiectomy was done show interesting changes with temporary return to normal five days before death. Autopsy showed neither coronary nor gross myocardial lesions.

It is thought that these observations may have some bearing upon the relative importance of increased intrapericardial pressure and anoxemia as the cause of such electrocardiographic changes.

*Pneumopericardium in the Treatment of Pericarditis with Effusion* By M H NATHANSON, M.D., Minneapolis Minn.

The literature contains comparatively few references to the therapeutic use of air in the pericardial cavity, only about twenty cases having been published. In the present report two patients with the exudative type of tuberculous pericarditis were treated first by the removal of fluid and later by the removal of fluid and injection of air. One patient has been observed for fifteen months and at the present time has a low grade tuberculosis of the peritoneal cavity. The other patient was first treated in 1923 and at the present time is in apparent good health although there is some enlargement of the heart.

A review of the literature and the experience in these two cases indicates the following:

- 1 Pneumopericardium can be carried out repeatedly without danger or discomfort to the patient.
- 2 The immediate result as evidenced by decrease in dyspnea and by increased diuresis is much better than that obtained by removal of the fluid alone.
- 3 As compared with simple removal of the fluid there is a distinct slowing in the return of the exudate with pneumopericardium.
- 4 The procedure permits a beautiful visualization on the roentgenogram of the pathology in the pericardium, the degree with which the sac is emptied by puncture, and the progress of the reaccumulation of the exudate.
- 5 Theoretically, the air, by keeping the inflamed surfaces apart, should reduce the mechanical irritation and also tend to prevent adhesions. This can only



be true in a degree as several cases in the literature which came to autopsy showed extensive adhesive pericarditis and both patients in the present report show roentgenograms indicating adhesive pericarditis

6 The possible curative effect of air on the exudate comparable to that which has been described in tuberculous peritonitis is suggested by the distinct improvement in the patient's general condition, the change of the fluid from hemorrhagic to clear, and the reduction in the number of tubercle bacilli in those cases in which they had been isolated.

*Liver Damage Incident to Diets Low in Protein* By M HERBERT BARKER, M D, Chicago, Ill

Dogs placed on a diet theoretically adequate in calories, minerals and vitamins but deficient in nitrogen gradually developed a fatty degenerative process in the liver. This fatty replacement became extensive if the diet was continued. A hypercholesterolemia developed early in the process and continued up until the animal's appetite began to fail. If protein was gradually added, the animal would usually recover. Repair of the liver has been extremely slow and functional tests and biopsies show extensive parenchymatous damage still present after six months.

*Weight Loss and Nitrogen Excretion of Obese Patients on Low Calorie Diets with High and Low Quantities of Protein* By ROBERT W KEETON, M D, and DOROTHY DICKSON (by invitation), Chicago, Ill

Obese patients weighing from 100 to 150 kgm were hospitalized and studied in the following manner. During a preliminary period of ten to fourteen days they were placed on diets containing 90 grams of protein and calories equivalent to their basal requirements. This allowed the patient's metabolism to become adjusted to this level of protein and his weight to become roughly stationary. He was then placed for a period of six weeks on a diet containing calories 30 to 48 per cent below his basal level and 90 grams of protein. This was followed by a second period of six weeks in which the diet contained 14 grams of protein and basal minus 30 per cent calories, and a third period similar to the first.

Eight patients were studied according to this plan. Data has been secured on twelve other patients who were unable to remain through such a long experimental period.

*Conclusions* 1 Patients living on diets containing 90 grams of protein and calories from 30 to 50 per cent below their basal requirements for six weeks, ten weeks and twelve weeks continued to lose weight uniformly throughout these periods.

2 Patients lost weight as well on diets containing 14 grams of protein, calories remaining the same as on diets with 90 grams of protein.

3 Weight loss occurred when the patients were in positive and negative balance. The fluctuations in weight loss were not correlated with fluctuations in nitrogen balance.

4 These patients requisition extra calories from the stored fat and so maintain their nitrogen equilibrium in the face of a severe caloric deficit.

5 The patients were more comfortable on the high protein diets.

*Study of Disorders of Duodenal Motility* By R. L. SENSENICH, M D, South Bend, Ind

Mechanical interference with normal duodenal motility, due to organic causes, produces retention and characteristic symptoms, and suggests that other phe-

nomena might result from temporary functional disturbances of motor activity. Roentgenologists have described various phenomena, said to be due to duodenal irritability, but without noting association of definite clinical symptomatology. Physiologists have in turn reported symptoms elicited by experimental irritation or stimulation of the undiseased duodenal wall. The study here reported was for the purpose of further investigating the association of common types of digestive discomfort with definite functional disorders of duodenal motility.

Patients have been observed over a period of years and possible organic causes have been eliminated. Association of certain symptoms with certain definite duodenal motor phenomena have been noted. These observations have demonstrated a tendency on the part of each patient to reproduce physical signs, subjective symptoms, and x ray findings, according to his individual pattern, whenever subjected to sufficient nervous stress or fatigue.

Characteristic clinical symptoms observed, varied from mild sensation of digestive malfunction to nausea and vomiting or violent cephalalgia. Disturbances of duodenal motility varied in degree and in the portion of the duodenum involved. Hypermotility changing points of irritability with delay, or spastic distal portion with duodenal distention and gastric retention were observed.

The diagnosis must be made by fluoroscopic observation continued throughout a sufficient period of time, when symptoms are present. This may be during transit of the barium meal or two to four hours later, when a small amount of barium must again be given to demonstrate the duodenal motility if the previous meal has passed. The significant diagnostic evidence is that the symptoms complained of are associated with the motor phenomena observed and are relieved when they cease. The use of small amounts of barium, suspended in water, best demonstrates the variations of duodenal motility. The recent work of Berg in bringing the surface of the mucosa into relief in roentgen studies gives promise of being of some help in differentiating organic duodenal conditions.

*Tumors of the Suprarenal Gland* By RALPH G. BALL, M.D. (by invitation) and LEONARD G. ROWNTREE, M.D., Rochester, Minn.

The various types of suprarenal tumors seen in the Mayo Clinic are and correlated with the cases in the literature. The series includes pseudohermaphroditism, hirsutism and virilism. The clinical manifest clear up subsequent to operation in many instances. On the other hand, from suprarenal failure has resulted in numerous cases following operation. Thus it may be possible to avoid in the future with the use of the new suprarenal cortical hormone.

Comparative study of the cases of paragangliomata of Mayo-Rowntree, Shipley-Pincoff and Porter and Porter reveals striking analogy in the history and clinical findings, in the operative results, and in the pathology of the tumors. Cases of continuous hypertension associated with medullary and cortical tumors with and without the changes in body configuration are reviewed. Twenty-nine cases have been collected from the literature in which there seems to be a relationship between suprarenal tumor and hypertension.

In relation to hypertension we must consider, first, that at least one active principle has been isolated from the suprarenal gland which has a tremendous effect on blood pressure. Second, that with tumors of this gland hypertension is extremely common and may be either intermittent or continuous. In at least one unoperated case, hypertension of the intermittent type has become continuous. Removal of the tumor has resulted in relief from hypertension in several instances. The lack of evidence of pathologic change in the suprarenal glands in

hypertension is no more striking than the lack of evidence of pathologic change in the pancreas in cases of diabetes mellitus. We are forced, then, to the view that there is a small and special group of cases of suprarenal hypertension, or that the suprarenals participate directly or indirectly in the pathogenesis of hypertension.

*An Analysis of the Lymphocyte in Pulmonary Tuberculosis* By B. K. WISEMAN, M D, and C. A. DOAN, M D, Columbus, Ohio

The characteristics of the blood-cells in pulmonary tuberculosis have recently assumed increased importance. Medlar, among others, has called attention to the significance of variations in the neutrophilic leucocytes and Sabin and Doan have emphasized the importance of the monocyte, its qualitative changes and the value of the numerical relationship existing between both monocytes and lymphocytes as expressed by the M/L index. Work done in our laboratory has indicated that certain qualitative changes occurring in the lymphocyte constitute criteria of a life cycle in that cell in many ways comparable to similar maturative phenomena in the other blood cells. This has led to the recognition of a lymphocyte formula that is rather constant in health. Utilizing these criteria, it has been possible to analyze the qualitative changes in the lymphocyte portion of the M/L ratio. Such studies in both clinical and animal tuberculosis indicate that the lymphopenia usually occurring in cases progressing unfavorably is due to an inhibition of the maturative forces in the lymphocytogenic tissues, and is reflected in the blood by an abnormal lymphocyte formula. Thus it has been found that while the M/L ratio is a valuable expression of an important numerical relationship between monocytes and lymphocytes, more complete information as to the progress of the tuberculous lesion can be adduced from an analysis of the qualitative changes in the lymphocytes and monocytes separately.

*The Problem of Tuberculous Reinfection* By R. G. BLOCH, M D, and B. F. FRANCIS, M D (by invitation), Chicago, Ill

The extensive use of the roentgen ray in the study of patients in our Chest Clinic has produced the material presented here. Our findings support the recently reported evidence of others for the theory that the infra-clavicular region of the lung is the most common location of the earliest lesion of active and progressive tuberculosis. This lesion is found on the x-ray plate before physical signs are manifest, often while symptoms are still very mild or even absent. The early lesion is more often found in the axillary half than in the medial half of the lung field, and varies from a few millimeters to several centimeters in extent. It also varies from the definitely circumscribed and homogeneous lesion to a diffuse one mottled in appearance. Occasionally excavation occurs and then it appears as an isolated cavity. The prognosis of this type of lesion varies. In many patients serial x-ray plates show a progressive disappearance even though no treatment is instituted, in others a long period of bed rest or collapse therapy is necessary. Of course, only a comparatively small proportion of patients are first seen in this early stage, most of them show more advanced disease on the roentgenogram. In these instances, when the apices are involved, the problem of the location of the early lesion arises. This question is discussed. Tuberculosis confined to an apex does exist and the pathological relationship between this type and the infraclavicular infiltration is also discussed.

In many of our patients who had had recent contact with tuberculosis, the early infiltration was found on the x-ray even though no symptoms or physical

signs of any kind were present. This led to the most important consideration of the study, the significance of the lesion from the public health standpoint, and the part played by exogenous reinfection in the etiology of the adult type of pulmonary tuberculosis. In our opinion it indicates first, that great attention should be paid to the isolation of patients with open tuberculosis from contact with adults, especially young adults, and second, that a good roentgenogram should be made of the chests of all contacts regardless of the presence or absence of symptoms or signs.

*The Copper, Non-Hemoglobinous Iron and Antitrypsin Contents of the Blood Serum in Disease* By ARTHUR LOCKE, PH.D, E R. MAIN, M.S., D O ROSBASH, B S (by invitation) and EDWIN F HIRSCH, M D, Chicago, Ill

The iron content of the hemoglobin-free serum of normal persons from whom food has been withheld for 12 hours varies from an average value of 0.9 microgram per cc. for young men to a value of 0.8 microgram for young women. Values of 1.4-1.5 microgram and above are found in the cord blood of newly born infants. Variable values are observed in disease. Conditions associated with fever usually are characterized by a lowered serum iron level, and conditions associated with a retention of bilirubin with an increased iron level. The high serum iron level associated with pernicious anemia becomes corrected following the administration of liver extract.

The copper content of the blood serum of young men appears to be lower and more variable than that of young women, ranging from a maximum of 0.9 to a minimum of 0.7 microgram per cc. The average Cu/Fe ratio for the blood serum of young women is 1.15/1, while that of young men appears to be 0.9/1. The copper level is slightly lower in uncomplicated bilirubinemia and in untreated hypothyroidism, markedly lower in the cord blood of newly born infants, slightly higher in pernicious anemia and untreated diabetes and markedly higher in cancer, pregnancy, leukemia, advanced tuberculosis, hyperthyroidism, and acute toxemia.

The capacity of the blood serum to inhibit the proteolytic action of trypsin is roughly proportional to the copper level. The antitrypsin content of potent extracts of the Rous chicken sarcoma becomes separated into the tumor inhibiting fraction, whereas the metal content appears to accompany the tumor inducing fraction upon isoelectric fractionation.

An increased Cu/Fe ratio of the blood serum may have the same significance in man that it has in certain marine forms accustomed to live in the lower levels of the sea, namely, an increased adaptation to oxygen want. A lag in the rate at which oxygen is supplied to the metabolizing tissues over the rate at which it has tended to become consumed would appear to have been present in all of the conditions in which the elevation of the Cu/Fe ratio was observed.

A preliminary study of oxygen want in rabbits maintained in an atmosphere containing carbon monoxide has indicated the existence of two distinct effects upon the Cu/Fe ratio. Rabbits responding with a compensatory increase in hemoglobin production and a parallel increase in the production of intracellular enzymes (as indicated by the greatly increased copper and iron content of their serums) appear to show almost no shift in Cu/Fe ratio. Rabbits unable to produce a compensatory increase in hemoglobin appear to show a rise in Cu/Fe ratio comparable to that observed in cancer and in acute toxemia.

*Blood Cultures of Apparently Healthy Persons* By ALLAN F REITH, PH D., and THEODORE L. SQUIER, M.D., Milwaukee, Wis

Blood culture studies have been made from a series of 293 apparently healthy persons all of whom were actively engaged in their usual occupation. Each

person was given a thorough physical examination including complete dental roentgenograms and examination of the prostate, sinuses, etc., for evidence of infection

After careful sterilization of the skin, blood was withdrawn from the cubital vein and 5 cc divided among three flasks of glucose brain broth to give dilutions of 1 100, 1 300 and 1 500. Cultures were examined after 7, 14, and 30 days, or as soon as growth became visible

Bacterial growth was obtained in 113 of 293 cultures. Cultures containing streptococci, diplococci, diphtheroids, *M catarrhalis*, *B coli*, or obligatory anaerobic rods were considered positive. Those containing only staphylococci were considered to be questionable. All others were regarded as negative. On this basis 65 cultures were positive (22 per cent), 28 were questionable (10 per cent), and 200 were negative (68 per cent)

In the series, cultures were made from 194 persons with definite evidence of focal infection. Of these 53 (27 per cent) gave positive and 124 (64 per cent) gave negative cultures. The remaining 99 persons were without demonstrable foci of infection. Only 12 of these (12 per cent) gave positive and 76 (77 per cent) gave negative cultures

Joint or muscle pain, including chronic infectious arthritis diagnosed clinically in 7, was present in 24 persons. Blood cultures were positive for streptococci or diplococci in 10, or 42 per cent, of these

A seasonal variation in the incidence of positive blood cultures from persons without demonstrable foci of infection suggests that acute respiratory infections may be responsible for some of the positive cultures

*The Intradermal Test in the Diagnosis of Tularemia* By LEE FOSHAY, M D, Cincinnati, Ohio

Intradermal injection of dilute suspensions of *Pasteurella tularensis* provokes a lasting unequivocal skin reaction in all patients with tularemia. The reaction is positive a week before agglutinins appear in the blood, occurring as early as the fourth day of disease. It is invariably positive in tularemia, it is never positive in normal controls, it is not positive in any other disease within the limited experience to date, it becomes negative only when convalescence is complete

Methods are described for preparing suitable antigens

Modifications of the reaction caused by persistent subcutaneous treatment of three cases with adequate antigens will be discussed

*A New Simple Method for Determining the Efficiency of the Peripheral Arterial Circulation* By GEORGE W. SCUPHAM, M D, and CARL JOHNSON, M D, Chicago, Ill

The instrument used is a sensitive air plethysmometer actuating a colored droplet of alcohol. It is made in various sizes and shapes to fit the digits or other parts of both extremities. Variations in volume of a digit with each cardiac impulse may be observed and measured. This volume change is shown by the pulsation of the droplet in a graduated 1 cc pipette

The character of the pulsation in normals is quite uniform under uniform environmental conditions. It is diminished or absent in thromboangitis obliterans and in arteriosclerosis depending on the severity of the occluding process

The instrument is of diagnostic value, and useful in gauging improvement in peripheral arterial disease. It is effectively and conveniently used on small members, hand, foot, or digit where the oscillometer fails

There are other possibilities to its usefulness. There are increased pulsations in aortic insufficiency, heart block and in peripheral vasodilatation. The increased pulsation of patients with fever is noteworthy.

A diminution in volume which occurs with respiration is interpreted to be the result of the aspiration due to inspiration and its effect on the venous return to the heart.

A method of permanently recording the movements of the droplet photographically on a moving strip of bromide paper is being devised so that accurate measurement of its oscillation and of the contour of the wave may be made.

*Arteriolar Studies in Patients with Hypertensive Heart Disease without Hypertension* By EMMET F. HORINE, M.D., MORRIS M. WEISS, M.D. (by invitation) and MARION F. BEARD, M.D. (by invitation), Louisville, Ky.

Biopsy specimens of the deltoid muscle were obtained from eleven individuals with cardiac enlargement but normal blood pressure and no other factor to explain the enlargement. Histological changes of the arterioles similar to those encountered in known hypertensives were found in all. Careful measurements of the arterioles of the specimens gave a ratio of wall to lumen from 1.131 to 1.070 similar to ratios found in known hypertensives and in marked contrast to the normal ratio of 1.2. Three patients are now dead and one autopsy was obtained in which a generalized arteriolar sclerosis was found. The frequency with which hypertensives show arteriolar lesions of the voluntary muscles is discussed. A diagnosis of "hypertensive heart disease without hypertension" may be confirmed by biopsy studies of the voluntary muscles.

*The Effects of Enclosing the Kidney in a Rigid Cast* By SAMUEL SOSKIN, M.D., and (by invitation) OTTO SAPHIR, M.D., Chicago, Ill.

Kidneys were aseptically enclosed in gauze and collodium casts, care being taken to avoid interference with the blood supply or ureters. When one kidney of an animal was so treated and the other kidney removed after several days or weeks, the animal invariably died. If however, the cast was removed from the one kidney before the other kidney was extirpated, the animal often survived. These results, together with some chemical studies indicate that the presence of such a cast incapacitates the kidney, but leaves little permanent damage detectable by ordinary means. Those animals which do not survive die without evidence of nitrogen retention in the blood. The manner in which the cast while in place, interferes with the kidney function, and the circumstances of the death of some of the animals after the cast is removed are suggestive of the existence of kidney functions with which we are not familiar.

*Chronic Lymphatic Reaction or Atypical Leukemia, Two Cases of Nine and Ten Years' Duration* By EDGAR T. HERMANN, M.D., St. Paul, Minn.

Origin and function of lymphocytes—probable defense rôle—classification of lymphatic reactions (slide)—exercise lymphocytosis (slide)—five types of lymphatic reaction (slide)—two cases under discussion (slide)—case of subacute aleukemic leukemia with hemorrhagic diathesis (slide) shown in contrast to previous two cases. The cases under discussion are both over seventy years of age and for ten years have shown no splenic or glandular enlargement of any kind. Total leucocyte counts have varied between 16,000 and 25,000 with a preponderant percentage, from 60 to 80, being lymphocytes. A study of the slides by Dr. Downey has failed to establish the diagnosis of chronic lymphatic leukemia.

*Leukemic Myelosis with Osteosclerosis* By J F BREDECK, M D, and D H STEPHENS, M D (introduced by David P Barr, M D ), St Louis, Mo

The report of Henck in 1879 of two cases of leukemia with bone changes has been followed by infrequent descriptions of generalized osteosclerosis of the skeleton accompanied by leukemic infiltrations of viscera with leukemoid peripheral blood picture in the presence of normal or slightly elevated total count. Two such cases in adults are described. The disease is apparently primarily an affection of bone, with compensatory myelogenous activity in extramedullary blood forming organs.

*A Comparison of the Newer Methods of Testing Renal Function* By J M HAYMAN, JR, M D, Cleveland, Ohio

The creatinine test of Rehberg, the urea clearance test of Van Slyke, the serum sulphate level, together with the phenolsulphonphthalein excretion, concentration tests, etc., have been run on a group of normal and nephritic patients, and the results scrutinized in the light of pathological findings and, where possible, with count of the number of glomeruli in the kidneys. It is concluded that by the use of several of the tests, particularly a combination of creatinine and urea clearance, a more detailed picture of kidney function can be obtained than by any test alone.

*Nature of Fibrillation and Flutter of the Heart* By L N KATZ, M D, and (by invitation) W A BRAMS, M D, Chicago, Ill

Several theories have been advanced to explain fibrillation and flutter of the heart. The two most widely accepted theories are the single pacemaker theory and the "circus movement" theory of Lewis.

Our experiments were planned to test the validity of these theories. For this purpose, electrograms of the exposed ventricles and of the exposed auricles were made during fibrillation and flutter. The ventricles were severed during the fibrillation and it was found that the two isolated chambers continued to fibrillate at approximately the same frequency as before. Similarly, in the case of the auricle, a crushing clamp was employed to completely separate the two chambers, with the same result.

These results are incompatible with the two foregoing theories.

*The Value of the Audiometer Test in Neuro-Syphilis* By LEON BROMBERG, M D, and ROBERT D SMITH, M D (by invitation), Chicago, Ill

Using a 2-A Westinghouse audiometer, curves were recorded on 100 unselected consecutive cases under treatment at the Public Health Institute. At the time these observations were made, the otologist was not informed about the clinical history or serologic findings of the patient. The test conditions were entirely standard for all patients, and were well controlled.

A careful review of these cases has shown a remarkable correspondence between defects in the upper vibratory tone range (1024 to 8192 double vibrations per second) and clinical or laboratory evidence of neurosyphilis. Considering the predilection which syphilis shows for the auditory nerve, the authors believe that the audiometer test offers highly useful supplementary evidence of invasion of the nervous system. The test is particularly valuable in cases where an examination of the cerebrospinal fluid is not feasible. Changes in the audiometer curve may also offer a useful index of response to treatment. The pathophysiology of the auditory defect is briefly discussed, and the authors present a few illustrative slides of characteristic audiograms and case histories.

*Organization of a Convalescent Serum Center and a Preliminary Report of Some of the Results in the Use of Several Convalescent Serums* By S O LEVINSON, M.D (by invitation), CLARICE McDOUGALL, M.D (by invitation), and WILLIAM THALHIMER, M.D, Chicago, Ill

A brief description of the organization of a serum center An outline of the necessary equipment and personnel and of the cooperation necessary for securing and distributing serum from patients recovered from poliomyelitis measles, chicken pox, scarlet fever, etc. A brief preliminary report of some of the results in prophylaxis and therapy

*Uric Acid Excretion as Influenced by Ultraviolet Light* By M G PETERMAN, M.D, Milwaukee, Wis

A group of thirty-eight convalescent children has been studied to determine the effect of sunshine, artificial quartz light, and indoor life on uric acid excretion of these children on normal and on high purin diets Urines were collected during these various periods and the total uric acid excretion determined. No significant variations were noted in the amount excreted under these conditions

*The Relation of the Pain of Peptic Ulcers to Gastric Acidity and Motility* By JACOB MEYER M.D, DOROTHY FETTER, M.D (by invitation), and ALFRED A STRAUSS, M.D, Chicago, Ill

Studies of epigastric pain in relation to gastric acidity and gastric motility were made in 24 patients Palmer's acid test was used and the gastric motility was studied by the balloon method.

In eight of fifteen patients with ulcer, pain occurred in response to the injection of hydrochloric acid and had no relation to the gastric motility

In six of the other ulcer patients, pain occurred synchronously with gastric contractions and not in response to acid stimulation

In nine cases of epigastric pain with no demonstrable lesion of the stomach or duodenum, six reacted positively to the acid test. Pain did not occur synchronously with gastric contractions in any of these cases

In four patients with gastric and duodenal ulcer, operation under local anaesthesia, direct application of dilute HCl in varying strengths, of 0.5 per cent, 1 per cent and 5 per cent resulted in negative pain response. Two of these were acid positive by the Palmer test, and two were definitely positive by the motility test.

The results indicate that hydrochloric acid is not responsible for the pain in all cases of peptic ulcer Hunger contractions or motility is undoubtedly an equally important factor The positive acid response in cases without intrinsic gastric and duodenal lesions, as in gallbladder disease, chronic appendicitis and gastric neurosis, would indicate the unreliability of the acid test as a diagnostic test of gastric and duodenal ulcer

*Negative Results with the Dye Method in Showing Blood Dilution* By WILLIAM H HOLMES, M.D, and J R. MILLER M.D, Chicago, Ill

Fifteen hundred cc. of 0.9 per cent sodium chloride solution were given intravenously to dogs, in which a normal blood volume value had previously been established. Blood volume determinations made immediately following the administration of fluid failed to show an increase in volume. Blood counts, hematocrit readings, and total protein determinations all indicated definite dilution.



*Chronic Idiopathic Hemoglobin Deficiency Anemia* By CHARLES H WATKINS, M D, and HERBERT Z GIFFIN, M D, Rochester, Minn

This paper is chiefly a consideration of the clinical manifestations of chronic hemoglobin deficiency anemia in which a definite etiological factor has not been discovered, as exemplified in a study of more than one hundred cases. The syndrome has been described in the literature under various names—chronic chlorosis, chloranemia, hypochromic anemia with achlorhydria and simple achlorhydric anemia. Some of the clinical considerations are as follows. The occurrence of achlorhydria, the presence of acid after histamine, the number of cases that show normal acidity or hyperacidity, the occurrence of menorrhagia, glossitis, stomatitis and paresthesias. It is clearly demonstrated that the clinical syndrome of chronic idiopathic hemoglobin deficiency anemia is not necessarily accompanied by achlorhydria.

*Blood Glucose Clearance Determination by a Microinterval Technique* By RICHARD M MCKEAN, M D, Detroit, Mich

Short-interval blood sugar studies were carried out on 100 normal subjects, mild diabetics, severe diabetics, and individuals suffering from hyperthyroidism, following the intravenous injection of 0.2 gram of glucose per kilogram body weight. They showed that the maximum blood sugar levels were reached within 3 to 7 minutes, and the 15-minute curve of assimilation was similar to that observed in the 3-hour period following oral administration of glucose. This method offers certain advantages over the oral method of glucose tolerance determination, chiefly in the amount of glucose administered, time required for the test, elimination of the enteral absorption factor, and added comparability of resultant curves.

*Eosinophilia Due to the Administration of Digitalis* By ABIGAIL E SMITH, M D, and STANLEY R BENNER, M D (introduced by D P Barr, M D), St Louis, Mo

A case of decompensated heart disease being treated with digitalis showed an eosinophilia of 25 per cent during administration of the drug. While the drug was withheld for 3 weeks the eosinophile count dropped gradually to 11 per cent. Upon resuming digitalis administration the percentage of eosinophiles rose promptly to 30 per cent. References to "digitalis eosinophilia" are found in the foreign literature. The phenomenon is discussed as a possible effect of vagus stimulation by the drug digitalis.

*Incidence of Organic and Functional Disease in Patients Whose Symptoms are Chiefly Abdominal* By S F ADAMS, M D, Rochester, Minn

This study was undertaken in order to find out how many people with "stomach trouble" as their chief complaint had organic disease.

These people were sufficiently concerned about their trouble to travel from three blocks to three thousand miles for advice. None of these people was referred by a physician.

The preliminary survey showed that 39 per cent had organic disease and that 52 per cent had no lesion of the stomach, biliary tract, pancreas, or bowel. A few were undiagnosed, there were 9 per cent in this so-called "indeterminate" group.

*Further Experience with Hyperinsulinism* By FRANK N ALLAN, M D, Rochester, Minn

At the meeting of the Central Society for Clinical Research in 1928, observations on three cases of hyperinsulinism were reported. Five additional

cases have since been studied. A review is given of investigations in the series of eight cases. The diagnosis is discussed from clinical and laboratory standpoints. In seven cases operation was performed. The results of medical and surgical treatment are presented.

*Prolonged Treatment of Polycythemia Vera with Phenylhydrazine* By EDGAR V. ALLEN, M.D., and HERBERT Z. GIFFIN, M.D., Rochester, Minn.

Detailed records are available regarding eleven patients with polycythemia vera who have been treated with phenylhydrazine since 1925. Survey of these data shows that in most instances the erythrocyte count can be maintained at a nearly normal level with small amounts of phenylhydrazine taken two or three times a week, or one day out of each week.

In three instances the patients had some difficulty in regulating the dosage with the result that the erythrocytes were occasionally too high or too low.

Four patients have been able to dispense with the use of phenylhydrazine with no return of the polycythemia after they have used the drug in the manner indicated above for periods of two or three years. The remaining patients have found it necessary to continue with the phenylhydrazine but have controlled the polycythemia satisfactorily from the standpoint of the number of erythrocytes and the symptoms usually associated with this disease.

There is no evidence from a clinical standpoint that the prolonged administration of phenylhydrazine does any damage to the organs of the body, but laboratory records were not available.

There is no evidence from our study that patients acquire a tolerance to phenylhydrazine, increasing doses of the drug are not necessary.

*Lipoid Nephrosis and the Nephrotic Syndrome* By E. G. BANNICK, M.D., Rochester, Minn.

This report does not embrace a review of the literature on this subject since this has been presented repeatedly and quite recently. It is a summary of the author's personal experience with this problem together with an analysis of practically all the adult patients presenting the nephrotic syndrome that have been studied at the Mayo Clinic between the years 1921 and 1930, inclusively. In most instances a fairly satisfactory follow up record has been obtained.

Special necropsy studies have been made in conjunction with Dr. N. W. Barker and Dr. D. L. Wilbur on the patients in this group that died at the clinic during this period. None of the necropsy cases represent the primary uncomplicated form of lipoid nephrosis in its strictest analysis, and yet in several instances the evidence of associated nephritis was so slight that very careful study was necessary to detect it. In these cases the pathological findings were practically identical with those of lipoid nephrosis that have been reported in the literature. The slight changes noted in the glomeruli, even in this group and by special stains, were not sufficient to warrant the assumption that so-called lipoid nephrosis is a form or stage of glomerulonephritis. However, the not infrequent association of the nephrotic syndrome with true glomerulonephritis, and the fact that the kidney even in pure lipoid nephrosis shows the same pathological picture and is the only organ which is consistently involved serve to prevent divorcing lipoid nephrosis entirely from renal diseases and considering it a general metabolic disturbance. On the other hand, the extra renal factors are so pronounced that pathological studies of the kidneys have thus far failed to offer a satisfactory solution to the problem.

From a clinical standpoint also uncomplicated lipoid nephrosis is rare, but there is not the paucity of cases for study that the pathologists note. At least

thirty cases have been studied at the Mayo Clinic during the years mentioned and have been so classified only after close analysis and strict adherence to the criteria that have been generally accepted. Most of these cases have subsequently improved and several have made complete recovery. A considerable number of borderline cases have been excluded, perhaps unnecessarily, because of a slight deviation from the normal criteria which have been rather arbitrarily established. The more evidence there is pointing to an associated glomerulonephritis, the poorer the prognosis becomes.

*Skin Tests in Chronic Ulcerative Colitis* By J C T ROGERS, M D (by invitation) and J ARNOLD BARGEN, M D, Rochester, Minn

To a series of normal controls were administered antigens prepared from the diplostreptococcus of chronic ulcerative colitis, streptococcus fecalis and the enterococcus by intradermal injection. Another series of fifty-six unselected patients with chronic ulcerative colitis were injected with the same antigens, intradermally, and the results recorded at intervals. Positive skin tests became negative during the administration of the specific antibody solution (concentrated serum) and coincident with the clinical relief of symptoms of colitis.

*The Tobacco Factor in Thrombo-Angitis Obliterans* By NELSON W BARKER, M D, Rochester, Minn

A study of the consumption of tobacco in 350 cases of thrombo-angitis obliterans as compared with that in 350 control cases disclosed that there were many more heavy smokers in the first group than in the second. However, the small but definite number of cases of thrombo-angitis obliterans in which the patients had never used tobacco or used only small amounts, raises the question of whether tobacco may be a direct etiologic factor in the disease. There is some statistical evidence to show that the disease tends to be more malignant if patients are heavy smokers, and that recurrences are fewer if the consumption of tobacco is decreased or stopped.

*On the Pathogenesis of Angina Pectoris with Special Reference to Its Association with Certain Severe Anemias. Report of a Case of Angina Resulting from Severe Posthemorrhagic Anemia* By MOSES BARRON, M D, Minneapolis, Minn

Exact pathogenesis of angina pectoris is still undetermined. Albutt's aortic hypothesis is falling into discard. Jenner's coronary basis is most likely the correct one. The anatomical basis, postmortem findings. Experimental support for coronary narrowing by Sutton and Lueth. Coronary spasm in ischemia. Anemia of muscle is the cause. Wenckebach's recent hypothesis of thinning and distension of muscular coats in arteriosclerosis. Angina pectoris is occasionally associated with severe anemias as reported by Herrick, Willius and Griffin, and others.

In all available literature no case is reported of angina associated with acute posthemorrhagic anemia. All anemias in the reported cases, whether pernicious or secondary in type, are chronic.

In this study the angina followed immediately upon a severe gastrointestinal hemorrhage.

Detailed report is given of a case with blood studies and electrocardiograms. With the disappearance of the anemia, the angina disappeared and the patient returned to his usual occupation.

*An Inorganic Standard for Hemoglobin Determinations* By A P BRIGGS, M D, St Louis, Mo

A solution which matches the acid hematin solution ordinarily used for colorimetric determination of hemoglobin has been prepared by adding small quantities of copper sulfate, chromium sulfate, and cobalt sulfate to a solution of ferric sulfate. The color matches acid hematin somewhat more closely than the Newcomer disc, has a known chemical composition, and its color value is permanent. Compared with acid hematin with the aid of a spectrophotometer roughly parallel extinction coefficient curves were obtained. The solution has been standardized by comparison with the acid hematin color of various bloods, the hemoglobin content of which had been calculated from iron determinations

*Acidity Patterns of Gastric Juice Obtained by Continuous Aspiration* By CHARLES L. BROWN, M D, and FRIEDRICH ENGELBACH, M D (by invitation), Ann Arbor, Mich

A method of continuous aspiration of gastric contents is described. Histamine was administered but no test meal was given. These studies are presented under two headings: (1) The acidity pattern of the gastric juice obtained from the same individual (normal) under similar experimental circumstances on different days. Ten or more observations are made in each case. It was found that the acidity curve may vary considerably in some respects at times. In general, however, the peak of the acidity curve was reached essentially at the same time after histamine administration during each observation. (2) The acidity pattern of the gastric juice obtained from the same individual on different days, but having graded doses of histamine. From these studies it would seem that the usual dose of histamine may be larger than necessary.

*Nutrition of Sick Children* By M G PETERMAN, M.D., Milwaukee, Wis

This investigation was a study to determine the food requirements of children sick in the hospital with chronic diseases, particularly tuberculous bone lesions. A particular interest was in the effect of diets with high fat, acid ash, or basic ash, and the influence of NaCl. Twelve children were studied for a period of nine months. All of the necessary procedures such as physical examination, blood counts, urinalysis, etc., were made. The patients were weighed and measured once a week.

The Sauerbruch-Hermannsdorfer diet has been recommended for tuberculous patients. This diet was reported to be a basic ash (but actually found to be an acid ash), low NaCl, high fat, high mineral, and high vitamin feeding. There was no striking clinical improvement in our study in any one period on any particular diet. The food requirements were found to be approximately those for normal, well, active children. From our study it appeared that the ideal diet for sick children was a basic ash, high vitamin, high mineral, attractive diet.

*The Vasodilating Effects of Alcohol* By GEORGE E. BROWN, M D, and EDWARD N COOK M D (by invitation), Rochester, Minn.

Various drugs have been tested for their vasodilating effect on the peripheral arteries. The most effective of them has been ethyl alcohol administered by mouth. This produces well-defined, prolonged vasodilation as measured by the surface temperature and the elimination of heat. Its action in a high percentage of cases of arteriosclerosis shows sharp vasodilation in the affected extremities.

*A Study of Proportional Heart Weights in Human Subjects* By JAMES G CARR, M D, and VICTOR LEVINE, M D (by invitation) Chicago, Ill

Hermann in 1925, on the basis of extensive experimental work, recommended a method for the preparation of hearts and the separation of the ventricles preliminary to weighing the ventricles. Following Hermann's method fifty human hearts have been prepared and weighed. This group includes hearts which are normal and hearts representative of the common varieties of cardiac disease. The relationships of the ventricles to each other, to the total heart weight and to the body weight have been determined and are presented.

*The Coexistence of Pernicious Anemia and Carcinoma of the Stomach* By H MILTON CONNER, M D, Rochester, Minn, and IVAR W BIRKELAND, M D (by invitation), Seattle, Wash

Eighteen cases are reported from the Mayo Clinic in which there arose the question of the coexistence of carcinoma of the stomach and pernicious anemia. In seven cases, the diagnosis of both pernicious anemia and carcinoma of the stomach in the same individual seemed reasonably certain, in five cases, the existence of carcinoma of the stomach seemed practically sure and that of pernicious anemia probable, in two cases, the diagnosis of carcinoma of the stomach depended almost entirely on roentgenologic examination, but the existence of pernicious anemia was probable, in four cases, the existence of carcinoma was reasonably certain and pernicious anemia was possible.

*Extract of Livers of Codfish in the Treatment of Pernicious Anemia* By H MILTON CONNER, M D, Rochester, Minn

Hansen and Stubb used livers of codfish in the treatment of pernicious anemia in five cases with good results. An extract was not so efficacious.

Connery employed an aqueous extract of livers of codfish in six cases with an effect comparable to that obtained with mammalian liver and its extract.

In this study an aqueous extract of codfish livers has been given to ten patients, two of whom had the liver extract only a few days because of intolerance, and one of whom was ambulant. Seven patients were studied in the hospital.

In an average of twenty-seven and six-tenths days in the hospital, the average of the erythrocytes of these seven patients increased from 1,710,000 to 3,390,000, the hemoglobin from 39.7 per cent to 67.6 per cent, and the leucocytes from 5,200 to 7,800. The increase in erythrocytes averaged 426,000 a week. The reticulocytes rose in one case to 30.4 per cent and the average height of the peak of the reticulocyte curve was 12 per cent of the total number of erythrocytes. The general condition was equally improved. Too few patients were observed to justify any conclusions regarding the effect on the nervous system, although there was some subjective improvement of three of the four patients who had subacute combined degeneration of the spinal cord, but this improvement was not marked during the short stay in the hospital.

*Some Qualitative Differences in the Action of Certain Commercial Digitalis Preparations* By G K FENN, M D, and N C GILBERT, M D, Chicago, Ill

In the course of an investigation on the effect of digitalis upon the coronary flow, it was observed that in addition to a difference in action on the coronary arteries, there was also a difference in the action of certain preparations on the conducting tissues, upon muscular irritability, and in the mode of death.

These points were worked out in a separate series of animals and are reported upon

*A Study of the Effect of Sodium Fluoride on the Dog's Thyroid* By LEO GOTTLEIB, M.D (by invitation), and SAMUEL B GRANT, M.D, St. Louis, Mo.

A relatively small number of dogs, with one lobe of the thyroid removed from each dog as control gland tissue, were given sodium fluoride either by mouth or intravenously, and the remaining lobe of the thyroid removed after various intervals and various dosages. The iodine content of the control gland and the gland after administration of fluoride showed no constant change, and there were no gross nor microscopic alterations in the structure of the gland.

L. Goldemberg and others have been able to produce goiters and a condition resembling myxedema in sheep, guinea pigs etc. Goldemberg has also used fluorine in the treatment of exophthalmic goiter.

*Changes in Haemodynamics under Physiological and Pathological Conditions* By W F HAMILTON, M.D, J W MOORE, M.D, and J M KINSMAN, M.D, Louisville, Ky

Various conditions which result in an increase in stroke volume of normal men and animals cause also a marked and quantitatively measurable increase in the intrathoracic blood volume. This is brought about through the increased left ventricular filling pressure on the elastic lung capillaries.

If the left ventricle is weakened through disease (congestive failure) an increased filling pressure is necessary to get a more or less adequate stroke volume (Starling's law of the heart). This increase in left ventricular filling pressure results in a marked and quantitatively measurable engorgement of the intrathoracic blood spaces. The stroke volume and the intrathoracic blood may, either one, approach the normal but the relation between them is strikingly different from the normal.

On recovery from decompensation the greatest and most constant change is a movement toward normal of the ratio between stroke volume and heart lung blood volume. The stroke volume, the minute volume and the heart lung blood volume may increase, decrease or remain unchanged.

The mean circulation time,—the time from the middle of the injection period to the center of gravity of the time concentration curve—is always increased in congestive heart failure, returning toward normal on compensation.

The total blood volume (vital red method) is almost uniformly increased by twenty per cent or more in congestive heart failure.

These considerations are illustrated by spot diagrams embodying the results of 100 experiments on man and 50 experiments on the dog.

*The Occurrence of Free Hydrochloric Acid in the Gastric Content of Patients with a Macrocytic Hyperchromatic Type of Anemia* By FRANK J HECK, M.D., Rochester, Minn

The occurrence of cases of macrocytic hyperchromatic anemias, other than pernicious anemia, in this country is unusual. The two cases reported here both were men, one aged 25 and the other aged 60. In both cases there was a severe degree of anemia with an average red count of 2,000,000. Test meal analysis showed the presence of normal amounts of free hydrochloric acid. In the first case there was rapid improvement when the patient was treated with six tubes of Lilly's liver extract per day. A reticulated erythrocyte response similar to that of pernicious anemia was seen in this case. Within five weeks

time the blood count had returned practically to normal. At the end of one year the blood count was normal, although the patient was taking only one tube of Lilly's liver extract per day. In the second case, on mixed treatment, using iron and liver or liver extract there was definite improvement. However, as soon as the liver or liver extract was discontinued there was a relapse both clinically and from the standpoint of blood count.

Morphologically these two cases showed all the findings common to pernicious anemia and it is important to point out that it is necessary to have other corroborative evidence for a positive diagnosis of pernicious anemia.

*Thrombo-Angitis Obliterans in Women* By BAYARD T. HORTON, M.D., and GEORGE E. BROWN, M.D., Rochester, Minn.

Thrombo-angitis obliterans is an occlusive vascular disease which usually affects men between the ages of twenty and forty-five years. The literature contains two acceptable reports of cases of this disease in females. Although more than 600 cases of thrombo-angitis obliterans have been observed at the Mayo Clinic the present report of eight cases is the first series of cases of women to be put on record, the diagnosis in two of these cases was proved pathologically. We know of no other disease in which common tissues are involved, such as veins and arteries, in which the disease is so nearly exclusively confined to one sex. A satisfactory explanation of sex distribution in this disease has not been determined.

*Effect of Liver Extract Intravenously on the Blood of Patients with Pernicious Anemia* By RAPHAEL ISAACS, M.D., S. MILTON GOLDHAMER, M.D. (by invitation) and CYRUS C. STURGIS, M.D., Ann Arbor, Mich.

Liver extract was given intravenously to two patients and four-hourly blood studies were made throughout the day and night for eleven days. The first evidence of a reticulocyte increase was in 28 to 43 hours, the calculated maximum reticulocyte percentage (on the basis of the oral method) was reached in 68 hours, the actual maximum in 88 to 108 hours, the return to normal was in 20 days after one dose. Although the dosage was 0.1 gram per kilogram body weight (total 6 grams in one patient, 10 grams in the other), the maximum reticulocyte rise was greater than the calculated response for 6 vials (27 grams) of liver extract daily by mouth or, for the period studied, 297 grams. The symptomatic improvement was very prompt and did not differ from the usual response to liver extract or ventriculin by mouth. These results were confirmed by 84 subsequent intravenous injections in 24 patients with pernicious anemia.

*Studies on Mercurial Diuresis. The Effect of Ephedrine in Enhancing the Action of Salyrgan* By CARL A. JOHNSON, M.D., and R. H. YOUNG, M.D. (by invitation), Chicago, Ill.

The authors have attempted to show by accurate animal experiments that ephedrine enhances the action of salyrgan by increasing the blood supply to the kidney through vasodilation of the kidney vessels and increased cardiac output.

Dogs anesthetized with Na barbital were used throughout the work. Both ureters were cannulated and simultaneous records of blood pressure and urine flow were made.

Ephedrine was injected at various intervals before and after the salyrgan injection. The optimum time for injection of ephedrine to promote maximum diuresis was about one-half hour after the salyrgan injection. Under these

conditions ephedrine increased the urinary output following the salyrgan from about 8 cc per half hour to 40 cc. per half hour

The authors feel that this method of inducing diuresis may be valuable in overcoming the reflex anuria often seen clinically

*Clinical Observations on Oxygen Therapy in Heart Disease* By W W HAMBURGER, M.D L N KATZ, M.D, and (by invitation) S H. RUBINFELD, M.D, Chicago, Ill

Six patients with heart disease were treated with 40 per cent oxygen eight times with clinical improvement three times Two of these patients had severe acute anoxemia associated with heart failure. Clinical improvement occurred twice in three oxygen treatments One good result was obtained out of five treatments in the four cardiac patients with long standing heart insufficiency and mild anoxemia One of the patients died in the oxygen room, and another soon after removal from the oxygen tent No striking diuretic action was noted such as reported by Barach.

The most constant changes, aside from the alteration of the composition of the arterial blood, noted in the patients while in the oxygen rich environment, were a slowing in heart rate and a diminution in the minute volume of respiration These changes also occurred in the control and non cardiac cases studied.

*Absorption from the Pleural Cavity of Dogs II The Lymphatic System* By WILLIS S LEMON, M.D, and (by invitation) GEORGE M HIGGINS, M.D, Rochester, Minn

A graphite preparation was injected into the pleural space of dogs This provided a means for the study of the functional lymphatic system of the dog's thorax.

The lymph nodes arrange themselves naturally into two main groups parietal and visceral The parietal group comprises the sternal, the vertebral, and the diaphragmatic nodes, the visceral group includes the mediastinal, pulmonary, tracheobronchial and tracheal nodes

The lymph drainage associated with these nodes may be grouped into six routes, two of which conduct lymph caudad and four cephalad. On the dorsal wall of the thorax lymph vessels pass caudad and empty into a large lymph node which lies dorsal to the anterior pole of each kidney Efferent vessels from these nodes lead directly into the cisterna chyli The cephalad lymph drainage along the dorsal median line is less easily demonstrated than the caudad one. Intercostal segmental collateral vessels are not constant, although they are far more nearly constant on the right than on the left side. On the right the collecting channels empty into the caudad tracheal group of nodes, on the left they empty into the mediastinal group of nodes

On each side of the sternum the lymphatic drainage system from the ventral pleura comprises a series of segmental channels which converge to form an extensive plexus lateral to the internal thoracic blood vessels beneath the transverse thoracic muscle Both pleural and abdominal lymph, draining the plexus, empty into the sternal group of nodes at the level of the first and second inter spaces The terminal efferent vessels from the sternal and the tracheal nodes usually become confluent and together join the thoracic duct or the right lymphatic duct.

*The Effect of Ischemia in Different Parts of the Body on Blood Pressure* By A. J MILLER, M.D, Louisville, Ky

The report consists of a discussion of the findings in experiments on animals in which the blood supply was partly shut off to the brain, voluntary muscles,



kidneys and other viscera, and the immediate and remote effects on blood pressure observed

*Scarlet Fever Streptococcus Antitoxin Its Use in the Treatment of Scarlet Fever* By A GRAEME MITCHELL, M D, and (by invitation) FRANK E STEVENSON, M D, and M V VELDEE, M D, Cincinnati, Ohio

The purpose of this study was to determine, if possible, the effect of scarlet fever antitoxin in treatment of an unselected group of cases. In order to do this, every other patient suffering from scarlet fever admitted to the Contagious Wards of the Cincinnati General Hospital was given antitoxin. That is, no selection was made according to the severity of the case. Complete protocols were kept and the analysis of these was made without a knowledge at the time of the method of treatment of the patient. The study will be presented by a lantern slide demonstration. In so far as this group of patients was concerned the study seemed to show that the antitoxin had a specific neutralizing effect because there was decrease in the duration of the rash, a change in the character and extent of the desquamation, and a reduction in the number of complications. For example, the number of complications was diminished in the serum-treated group in relation to the expected number of complications as judged by their frequency in the control group. There was a large percentage of serum sickness but this occurred twice as frequently in patients who had previously received injections of horse serum in some form.

*Observations on the Urinary Cast Count During Administration of Salyrgan* By CHARLES L BROWN, M D, and (by invitation) FRIEDRICH ENGELBACH, M D, Ann Arbor, Mich

In recent years certain mercurial compounds have been used rather widely as diuretics. The question is raised as to whether these substances in clinical doses are harmful to the kidneys. The urinary cast count offers a possible index to nephrotoxicity. This type of observation was carried out during the administration of salyrgan as follows: (1) to normal rats in graded doses, (2) in clinical doses to cases of cirrhosis of liver, with ascites but no evidence of renal disease, and (3) in clinical doses to one case of subacute nephritis with edema (nephrosis?). In the rat, salyrgan in the dose corresponding to the human clinical dose caused no appreciable change in the urinary cast output. Larger doses may cause an increase in the cast output, which tends to be temporary, if the dose is not too large. In some of the cirrhosis cases there was slight increase in the cast count which tended to be temporary. In the case of nephritis the cast output was not changed appreciably.

*Essential (Primary) Hypertension A Clinical and Morphological Study of 375 Cases* By FRANCIS D MURPHY, M D, and (by invitation) JOHN GRILL, M D, Milwaukee, Wis

Essential (primary) hypertension may be associated with such a variety of clinical and morphological changes that successive stages of this disorder may appear to be separate diseases. From a clinical and histological study of 375 cases of essential hypertension an attempt is made to show that the various syndromes and vascular lesions observed are manifestations of the same process varying in its intensity and its speed of advancement. The cases are arranged into three groups representing three consecutive stages of the disease. In group 1 are the earliest cases illustrating the functional stage, in which symptoms are unrelated to vascular lesions. An accident or an infection caused death in this group before arteriosclerosis became well established.

Group 2 or the arteriosclerotic stage embraces the cases in a more advanced stage in which arteriosclerosis is a prominent feature, death resulted usually from arteriosclerotic lesions

Finally, group 3 includes the cases with a rapid and stormy course ending fatally within a short time. Histologically, these cases are distinguished by advanced arteriosclerosis with necrotic lesions of the arterioles of the kidney and other organs. Group 3 may be classed as the arteriolonecrotic group. Group 1 and 2 are commonly designated as 'benign' hypertension and group 3 as "malignant" hypertension.

There are 43 cases in group 1, 303 in group 2 and 29 in group 3. A statistical analysis is given in tabular form of the causes of death, complications, heart weights, and age incidence in each group.

Histologically, minimal vascular changes are observed in group 1, the functional group. In group 2, there is a decided arteriosclerosis in medium sized arteries, while in smaller arteries there is a localized arteriosclerosis leading to ischemia and slow atrophy of one or other organ. The arteriosclerosis in group 3 is more diffuse and intense involving more definitely the smaller and smallest arteries and leading to necrotic lesions of the afferent glomerular arterioles as well as of arterioles in other organs.

Although, clinically the chief features of group 3 are fairly uniform and constant, histologically the arterial lesions, especially in the kidney, may vary considerably. Whether the renal lesions represent the results of a toxic or inflammatory influence added to a kidney already damaged by arteriosclerosis, or whether they indicate the effects of arteriosclerosis alone is a disputed question. The interpretation of these lesions and their correlation with the clinical picture are presented. It is concluded that there is no fundamental difference between the three groups, but that the numerous changes observed represent various degrees of intensity of the arteriosclerotic process.

*The Diagnosis of Early Tuberculosis in the Strict Sense of the Word* By J. A. MYERS, M.D., Minneapolis, Minn.

This paper is based upon results of tuberculin testing and making x ray films of the chests of students of nursing and medicine. On admission to such schools approximately 35 per cent react positively to the tuberculin test. After taking services in tuberculosis the incidence of positive reactors increases markedly. Along with this increase a considerable number of those who previously reacted negatively to the test but became positive during or after tuberculosis service have developed demonstrable tuberculous lesions in the lung parenchyma and cervical lymph nodes. Pleurisy with effusion is a rather common finding. In this group of students we can speak of early diagnosis in the strict sense of the word. Not one of these young adults has developed acute rapidly progressing tuberculosis but all have reacted to the infection and the disease in about the same manner as children who first become infected. They respond well to treatment. Without the tuberculin test and the x ray film examination most of these cases would not have been diagnosed. In other words the lesions are too nearly minimal to be detected by our other methods of examination. This study brings to light the fact that in our schools of medicine and nursing we are transmitting tubercle bacilli to the majority of the students who have never before been infected. An alarmingly high percentage fall ill with the disease.

*The Role of Hemolytic Streptococci in Arthritis* By I. PILLOT, M.D., Chicago, Ill.

Evidence is introduced that arthritis may be due to hemolytic streptococci of various types the *Streptococcus scarlatinae*, *Streptococcus erysipellatis*, and

the *Streptococcus epidemicus* of septic sore throat. Arthritis indistinguishable from acute rheumatic fever may develop during the third or fourth week after the onset of a streptococcus sore-throat. This interval suggests the development of an allergic state to the streptococcus. For this conception some experimental work is reported. Rabbits receiving 0.01 cc of living *Streptococcus epidemicus* at weekly intervals did not develop arthritis until the third or fourth injection. Broth filtrates and vaccines apparently did not sensitize the rabbits to arthritis from minute doses of living streptococci. In the human subject the sensitizing streptococci are derived from the focus in the throat, which in our cases yielded the streptococci in swab cultures from the tonsils. The *Streptococcus epidemicus* can be readily differentiated from other hemolytic streptococci and the appearance and disappearance of this organism can be correlated with the arthritic manifestations. The removal of tonsils harboring this streptococcus often led to relief of arthritic symptoms.

*Some Observations of the Effects of Hog Stomach Extract (Ventriculin) on Gastric Secretion and Gastro-Intestinal Symptoms* By LEON SCHIFF, M D, and (by invitation) GEORGE BURGER, M D, and TOBA TUHL, Chicago, Ill

The effects of the administration of ventriculin on the symptoms of a number of patients suffering with achlorhydria were noted. Repeated determinations were made of the acidity and pepsin content of the gastric juice in these individuals.

The effects on the gastric juice of the administration of a single dose of liquid extract of ventriculin were also noted in healthy individuals as well as in those suffering with gastro-intestinal disturbances. These effects were compared with those obtained with the alcohol meal, oatmeal gruel, and following the ingestion of histamine.

*The Use of Follicular Hormone in Involutional States* By ELMER L. SEVRINGHAUS, M D, Madison, Wis

The manifestations of the menopause include not only the well known vasomotor symptoms but also some features which are classed as neurological or psychiatric. In a group of 30 cases with various aspects of the climacteric 14 reported paresthesias as troublesome. In 17 cases insomnia was complained of, with no other evident explanation. Half of the series had definite psychotic symptoms, which varied from increasing nervousness to the typical involutional syndrome including despondency, subjective inadequacy for the usual tasks, emotional lability, suspicious attitude, and suicidal tendencies. These cases were seen early in the course of the mental difficulty. Of the group of 15 there were 13 who received follicular hormone as the chief material in treatment. In two cases the improvement was questionable, but in 11 there was no doubt of marked relief within a few days. Most of these cases were observed for a long enough period to give assurance of maintained help by the hormone therapy. The paresthesias and insomnia have been similarly benefited.

The frequency of obesity in the group of 30 is low, only 11 cases. Several of the obese patients had been overweight for many years. Obesity does not seem to be more frequent in the older members of this group. Hypertension was found in only 10 of the cases. The typical hot flashes were seen in 27 patients, and in two others the vasomotor instability was reported as chills of short duration.

The age range is from 32 to 56 years. The 10 cases with age 41 or less include 6 with artificial menopause, 1 with a pseudo-thyrototoxic picture, and 3

which are apparently cases of hypofunction of the ovaries. In 2 of these 3 the use of follicular hormone has re-established regular menstrual cycles and given relief from the premature involutional psychosis which was the chief complaint.

The dose range of hormone which is effective is from 10 to 40 units daily by hypodermic route, or from 25 to 50 units each night by vaginal pessary. The commonest dose has been 20 units daily, given in one injection. Patients make the injections readily, using amniotin or theelin.

*Experimental Treatment, with Stramonium, of Patients Exhibiting Evidences of Thyrotoxicosis* By R. L. SENSENICH, M.D., South Bend, Ind.

The favorable effect of atropine upon patients with exophthalmic goiter has been reported by many observers.

Stramonium was suggested for this study because of the freedom from objectionable effects when given in very large doses over long periods of time in certain other conditions, notably the sequellae of encephalitis.

The drug was given to eight thyrotoxic patients, none of the class of adenomas with hyperthyroidism. Basal metabolism readings, before treatment, ranged from plus twenty two per cent to plus fifty-eight per cent. All had lost weight and exhibited tachycardia and marked tremor, with other diagnostic evidence of toxic thyroid states. Seven of these patients were markedly improved, metabolic rates being reduced and gains in weight being recorded. Nervousness was relieved and tremor was diminished or controlled. Heart action was slowed to some extent (eighty to ninety in recumbent rest), but not controlled to a degree comparable to the improvement otherwise attained. One patient in the menopause exhibited such unpleasant cutaneous "flush" when the drug was administered that it was impractical to continue. Some described subjective eye symptoms if they read long, but there was no evidence of increased intra-ocular tension.

The stramonium was given in the form of a preparation of the leaves, in doses varying from  $7\frac{1}{2}$  to 15 grains daily. No other medication was given. No bad effects were observed during more than two months of continuous administration in maximum dosage.

*Summary* Improvement was observed in thyrotoxic patients, without bed rest, under stramonium treatment. This improved state was maintained throughout a period of four months following cessation of treatment. Some acceleration of the pulse rate continued, although diminished in frequency under the treatment and followed by gradual slowing after its discontinuance. Judging from reports of results obtained under atropine treatment, the use of stramonium seemed to possess some advantages in results and possibility of continuous administration in effective dosage.

This series of cases is too limited to report for the purpose of arriving at any conclusions and is given only as work in progress. The study is being continued with the thought of using stramonium in conjunction with other measures.

*The Endogenous Uric Acid Metabolism in Polycythemia Vera* By S. A. SHELburne, M.D., Dallas, Texas, and (by invitation) R. F. HANZAL, M.D., Cleveland, Ohio

The authors have measured the daily endogenous uric acid excretion in a case of polycythemia vera daily during a period of four months. These findings have been correlated with the clinical condition of the patient, the changes

in the blood cells and the blood chemistry before, during and after two periods of treatment with phenylhydrazine

This patient had a persistently great increase in the endogenous uric acid excretion, which is the condition one would expect if this disease is due to an increased formation of red blood cells. It is well known that increased erythrocyte formation is associated with increased uric acid excretion. We also observed some changes that indicate that phenylhydrazine has a depressing action on erythropoiesis.

*Available Carbohydrate and the Blood Sugar Level in the Treatment of Older Diabetics with Cardio-Vascular Disease* By SOLOMON STROUSE, M D, SAMUEL SOSKIN, M D, LOUIS N KATZ, M D, and (by invitation) S H RUBINFELD, M D, Chicago, Ill

There have been many reports upon the deleterious effects of insulin shock upon the myocardium. It is not known whether the harmful action is due to the hypoglycemia or to a direct action of the insulin, or a component, upon the heart muscle. Nor is it known whether the continued administration of insulin as a therapeutic procedure, in doses small enough to avoid hypoglycemia, carries with it a similar menace to the cardiovascular system of older diabetics with cardiovascular disease. Our results show that the therapeutic use of insulin, in such patients, may be accompanied by significant cardiovascular injury. They further indicate that the blood sugar level and the carbohydrate available to the damaged myocardium are important factors in this connection.

*Specificity of Certain Strains of Streptococci for Endocardium* By THEODORE L SQUIER, M D, and (by invitation) NORBERT ENZER, M D, and ALLAN F REITH, Ph D, Milwaukee, Wis

Certain strains of green producing streptococci produced endocardial lesions in a high percentage of rabbits after intravenous injection. Other strains apparently culturally similar consistently failed to do so.

During the past six years 539 rabbits have been injected intravenously with 147 strains of streptococci isolated from various foci of infection. With 25 of these strains, gross heart lesions developed in one or more rabbits after intravenous injection, and were found in 79 of a total of 164 rabbits thus injected. The remaining 123 strains failed to produce heart lesions in any of 375 rabbits injected.

The production of endocardial lesions by two strains of the green producing streptococci has been demonstrated through four and seven animal passages respectively. With each passage the streptococci were recovered from the heart blood, cultured in glucose brain broth and re-injected.

With one strain isolated from the blood culture of a patient with subacute bacterial endocarditis, endocardial lesions have been produced from subcultures after various time intervals. Finally after thirteen weekly blood agar transplants, single cells were isolated and subcultures from each of three cells produced typical vegetative endocarditis.

*A Clinical and Roentgenological Study of the Significance of Palpability of the Liver* By CARROLL W OSGOOD, M D, and J E HABBE, M D (introduced by T L Squier, M D), Milwaukee, Wis

Fifty-three patients without clinical evidence of hepatic disease in whom the liver was found to be palpable, were studied in comparison with a control group of fifty-three patients with non-palpable livers. Roentgenological determinations were made of the size of the right lobe and the relation of the inferior

border of the liver to the costal margin. The level of the liver border was also determined by palpation, and observations made of the thickness and relaxation of the abdominal wall, the depth of inspiration, and the weight and build of the subject. Liver function was determined by intravenous dye tests

Little correlation was found between palpability and liver size. The average x ray size of the palpable livers was no greater than that of the non palpable ones in the control group. Practically all of the livers observed descended below the level of the costal margin with deep inspiration, and the non palpable ones moved downward about as far as did the palpable ones. None of the livers studied exceeded the statistical limits of normal. While obviously such factors as the size of the liver, its relation to the costal margin and the tenseness of the abdominal wall, must in some instances affect palpability, no important influence was noted in this study. In the palpable group no appreciable impairment of liver function could be demonstrated by intravenous dye tests. Possible differences in firmness between the livers of the two groups could not be observed but the results obtained suggest that palpable livers are not necessarily pathological.

*Gastric Achylia (Neutral Red Studies)* By M. H. STREICHER, M.D., Chicago, Ill.

In recent years it has been claimed by some investigators that neutral red possesses a property similar to that of histamine as a stimulant of gastric acidity. Others considered neutral red of value in the differential diagnosis between true and false achylia.

We have attempted in this work to study the function of neutral red in gastric acidity determinations. For this purpose we obtained a series of ninety-six cases and classified them into three groups based largely on time of appearance of the dye in gastric contents. Gastric contents were collected every fifteen minutes for two hours and studied.

Briefly our results show that neutral red may be used to advantage as a test of gastric function and that neutral red possesses little or no property as a stimulant of gastric acidity. In differentiating between false and true achylia it may be employed because a prolonged period is necessary to perform the test and not because the dye is employed in the execution of the "neutral red test."

*The Blood Pressure Curve in Untreated Cases of Hypertension* By DON C. SUTTON, M.D., and (by invitation) SAMUEL LANG, M.D. Chicago, Ill.

During the past four years a special study has been made of cardiac patients with hypertension, in the Cardiac Clinic at Cook County Hospital. Of this group, sixty-four patients, forty-four of whom were men, have been segregated for study as normal controls. They have received no medical treatment except occasional digitalis, and have been given standard directions, with the exception that diets have been adapted to the individual needs of the patient. These patients have been studied for an average of fourteen weeks, the longest period being seventy-two weeks. Observations have been made on variation of both systolic and diastolic blood pressure in this group and from this study we have evolved a standard method of control for use in our clinic. Further studies of various methods of treatment will be made. As an illustration of this method of control, it is compared with a series of cases treated with potassium thiocyanate.

*Some Clinical-Pathologic Correlations of Adherent Pericarditis* By HARRY L. SMITH, M D (by invitation), and FREDRICK A. WILLIUS, M D, Rochester, Minn

This study comprises 144 cases of adherent pericarditis coming to necropsy at the Mayo Clinic. The pericardial lesions were not minimal but in all instances were well marked examples of the disease.

*Age and sex* The great majority of the cases occurred in the fourth, fifth, sixth and seventh decades of life, 108 cases (75 per cent). The youngest patient was two years of age and the oldest patient 85 years of age. The average age was 48.8 years.

A marked dominance in males occurred, 100 cases (69.4 per cent), while the female incidence was only 44 cases (30.6 per cent).

*Pathologic groups* The cases were divided into three groups according to the character of the adherent process. Complete sac obliteration 53 cases (36.8 per cent), partial sac obliteration 79 cases (54.9 per cent) and parietal adhesions 12 cases (8.3 per cent). Calcification of the pericardium occurred in 11 cases (7.6 per cent).

*Heart weights* Well marked cardiac hypertrophy was the rule, the average heart weight of the entire group (weights recorded in 106 cases) was 478.1 grams (average normal weight 290 grams).

The greatest heart weight occurred in those cases with partial sac obliteration where the average was 506.5 grams. Where the pericardial sac was completely obliterated, the average heart weight was 472.7 grams and where parietal adhesions occurred the average weight was 251.2 grams. One heart in this latter group weighed 1925 grams inclusive of the pericardium, a chronic tuberculous pericarditis, which was excluded in this average computation owing to its great variation from the other group components.

*Associated pathologic cardiac changes* Associated pathologic changes in the heart occurred in 77 cases (53.5 per cent). Their occurrence, in order of frequency, was as follows: Coronary sclerosis, 31 cases (21.5 per cent), rheumatic heart disease with mitral stenosis, 25 cases (17.4 per cent), hypertensive heart disease, 11 cases (7.6 per cent), rheumatic heart disease with aortic insufficiency, 6 cases (4.2 per cent) and aortic syphilis, 4 cases (2.8 per cent). The heart weights of these groups are compared.

*Associated intrathoracic pathologic changes* Twenty-five cases (17.3 per cent) had associated intrathoracic disease which is discussed.

*Mode of death* The heart was solely responsible for death in 57 cases (39.5 per cent), while causes entirely independent of the heart caused death in the remaining 87 cases (60.5 per cent). This data is discussed in relation to the various forms of associated cardiac disease. The average heart weight of the patients dying of heart disease was 625.7 grams, while those dying of other causes had an average heart weight of 411.6 grams. The age factors in relation to death are included in the discussion.

*Parenteral Use of Liver Extract in the Treatment of Anemia* By PAUL J. FOUTS, M D (by invitation), and L. G. ZERFAS, M D, Indianapolis, Ind.

Eight patients with anemia, six of whom had primary pernicious anemia, were treated with liver extract administered intravenously. The initial red blood cell levels in five of the cases which showed a reticulocyte response ranged between 1.13 and 3.65 million per cubic millimeter of blood. The maximum reticulocyte response was reached on the fifth day, following the first injection of an amount of liver extract derived from one hundred grams of whole liver.

One patient with hemolytic jaundice failed to respond satisfactorily to two blood transfusions, to liver extract derived from 600 grams of liver given daily for thirty days, or to 0.18 gram of metallic iron (iron ammonium citrate) administered daily for fourteen days. When liver extract derived from 100 grams of liver was given intravenously, a typical response of the reticulocytes occurred, followed by a satisfactory return of the red blood cells to a normal level.

The comparative potency of liver extract given by injection is discussed, with certain indications for the use of this method.

*The Effect of Diacetone Alcohol on the Liver of Rats* By HADDOW M. KEITH, M.B., Rochester, Minn

Redistilled diacetone alcohol was administered by stomach tube to white rats, in doses of 2 cc. per kilogram body weight. Pairs of rats were sacrificed at intervals of from one hour to 60 days. Sections of liver were fixed for 24 hours in a Zenker formol solution, stained with hematoxylin and eosin and with eosin azure II. Four animals were sacrificed as controls.

A distinct drop in the blood hemoglobin and in the red cell count was noted in 24 hours. This persisted for a period of four to five days. On the sixth day both were again approximately normal.

Examination of sections of the liver showed an early increase in lymphocytes and histocytes, followed by vacuolization and granulation of the hepatic cell cytoplasm commencing in the portal zone. This latter was accompanied by a decrease in the histocytic reaction. The maximum hepatic cell injury occurred within 24 hours when the entire lobule was involved. Recovery was first evident at 48 hours, mainly about the central veins, and within 96 hours was relatively complete. At seven and fourteen days, however, there was still slight cytoplasmic granulation in the portal areas, with definite histocytic reaction still present. At twenty-one days, the Kupffer cells were exceedingly abundant, and nests of histocytes were very numerous. The hepatic cells were normal. In thirty-five days recovery was complete.

*On the Value of the Electrocardiograph as an Aid in the Diagnosis of Adhesive Mediastino Pericarditis* By JULIUS JENSEN, M.D., and (by invitation) MILTON SMITH, M.D., and E. D. CARTWRIGHT, M.D., St. Louis, Mo

In an analysis of fifty normal individuals absence of shift with position in the complexes of the electrocardiograph occurred so frequently as to suggest that the Dieuade sign is of little value in the diagnosis of adhesive mediastino-pericarditis.

*Comparison of Serum Inorganic Sulphates and Blood Urea Clearance in Renal Insufficiency* By E. G. WAKEFIELD, M.D., M. H. POWER, PH.D. (by invitation) and NORMAN M. KEITH, M.D., Rochester, Minn

The determination of serum inorganic sulphates as a renal functional test has been compared with urea clearance. There is a fairly close correlation between the two as a measure of early renal insufficiency. Previous studies have indicated that serum inorganic sulphate is usually increased (1) before there is retention of either urea or creatinine, (2) before there is lowered excretion of phenolsulphonephthalein and (3) in about half the subjects studied sulphates were elevated in the serum before the kidney had lost its ability to concentrate urine to a specific gravity of 1.025 or more in the concentration test, also, the kidney might be unable to concentrate urine to 1.025 and the sulphates would be within the normal range of concentration. These tests do not replace, but supplement other tests of renal functional activity.



*The Determination of Inorganic and Conjugated Sulphates in Urine and Blood Serum* By M H POWER, PH D (by invitation), and E G WAKEFIELD, M D, Rochester, Minn

The method involves the preparation of trichloroacetic acid filtrates of urine or of serum. From these the sulphate is precipitated by the addition of a solution of benzidine in acetone. The resulting benzidine sulphate is centrifuged down, washed, and quantitatively oxidized by means of concentrated sulphuric acid and a known amount of potassium dichromate. Iodimetric titration of the residual dichromate then supplies the data from which the  $\text{SO}_4$  content of the sample may be calculated. The method has been found satisfactory for amounts ranging from 0.03 to 4.0 mgm of  $\text{SO}_4$ .

Conjugated sulphates are determined in an exactly similar manner after preliminary hydrolysis. The technique developed has been found applicable to the study of the ethereal sulphates in urine as judged by comparison with the results of the Folin gravimetric method. Similar studies of blood serum indicate the ethereal sulphates are present, but not in the amounts which have previously been reported.

# STUDIES ON THE EFFECT OF THE ACTION OF DIGITALIS ON THE OUTPUT OF BLOOD FROM THE HEART

## I THE EFFECT ON THE OUTPUT OF THE DOG'S HEART IN HEART-LUNG PREPARATIONS<sup>1</sup>

BY ALFRED E. COHN AND J. MURRAY STEELE

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A satisfactory solution of the problem concerning the effect of giving digitalis, especially on the volume output of the heart, but of other problems concerning its action, is still outstanding. Difficulties in the way of complete understanding depend, it seems, on the circumstances in which digitalis is administered. Among these are the species of animal which is studied and the state of the heart, whether healthy or diseased. The value of digitalis as a therapeutic agent, being on many accounts a constant source of concern, is viewed as being involved in these issues. Discussion is often confused, action of the drug and the beneficence of that action being regarded as the same things. This inference need not necessarily be correct. The pulse rate may be lowered, the blood pressure raised, the excretion of water unchanged, the volume output of blood from the heart either decreased or increased, the effect of not one of these actions can supply a clue, *a priori*, to whether giving this agent is advisable. In terms of no one of these functions can its value or benefit be described. The situation is different in an infectious fever. Barring the artificial lowering of temperature as a single effect without influence on the general course of the illness, defervescence is usually, and perhaps justly, regarded as a sign of value.

If the success of an agent cannot be measured in terms of a "test" of its efficacy, how can it be measured? Is it necessary to rely on the report of its effect by patients or is there something in the totality of their demeanor which permits the drawing of a relevant inference? In whatever way this phase of the matter is decided, an outstanding consideration is this, that a judgment is desirable which is not dependent on the measurement of some function, involved no doubt in the total action, but yet not characteristic of a result regarded as satisfactory. In the case of giving digitalis for example, patients may, conceivably, report

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<sup>1</sup> A preliminary report of these researches was read before the Association of American Physicians (Trans. Assoc. Am. Phys., 1931, xlvii, 71.)

"improvement" on taking the drug Under these circumstances, if we associate with improvement increase, for example, in the volume output of blood from the heart and learn, unfortunately, that decrease has taken place, we face a dilemma, the output has decreased but the patient is better To know the volume output is, for the present, an inadequate guide The problem is not solved by turning from methods of analysis to others much coarser and not susceptible to detailed examination, that way would be the negation of method Further study is needed to attempt to discover a form of analysis which tells us what we desire to learn, perhaps a method which measures the effect of an agent on the behavior of the entire individual Knowledge may consist, obviously, in terms either of the whole or of one of its analyzed parts But the balance between what the part can tell and what the whole describes is delicate, what must, it seems, not be upset is judgment of the whole, in favor of a part Digitalis, in short, should be given if patients improve, even if, were this the case, the volume output of blood decreases as the result of taking it

The fruitful experiments of Harrison and Leonard (1) have made it clear that the volume output of blood from the heart can be reduced by digitalis Important as this information is, suggesting, as it does, a new point of view from which to weigh the action of digitalis, a wide generalization based on this phenomenon seems inadmissible For there may be, as has turned out to be a fact, a difference between hearts which are normal in size and hearts which are enlarged<sup>2</sup> If an effect of digitalis is to decrease the size of all hearts, small as well as large, this result may still make a difference in the subsequent output of hearts initially normal and of hearts initially enlarged But aside from size, there might be involved, in enlarged, diseased hearts, an additional factor That this might be the situation was suggested in a report of experiments which confirmed those of Harrison and Leonard

In the experiments of Cohn and Stewart (2), decreased output of the normal heart as a consequence of the action of digitalis was described as a fact, associated with decrease in the size of the heart The effect on output was ascribed to two factors, one on the size of the heart which was designated tone, and a second, an effect on contraction Of the two, the effect on size predominated This explanation Dock and Tainter (3) regard as unsatisfactory because it failed to take into account "all the known and established actions of the drug on the circulation and does not correlate the latter with factors determining cardiac output" They urged the view that in any judgment of the action of digitalis on the vol-

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<sup>2</sup> The word enlarged is used, not to obscure the difference between dilatation and hypertrophy, but to suggest that of these conditions the one which is present is not always known The two aspects of enlargement can, furthermore, not always be separated

ume output it is not enough to study only the behavior of the heart, its effect on the blood vessels must also be estimated. The fact that in their experiments "a fall in venous pressure regularly accompanied the diminished cardiac output" they regard as "convincing evidence that the change in blood flow was a result of peripheral, and not cardiac, actions of the drug" (Conclusion 3) (3). Tainter and Dock (4) showed later that "the fall in right auricular pressure, after giving digitalis to dogs with the hepatic circulation intact, was due to diminished venous return flow, and ultimately to an accumulation of blood in the splanchnic or portal region as a result of obstructed hepatic outflow (hepatic vein constriction)".

This mechanism adequately explains the diminution in cardiac output after digitalis, since a diminished venous return must result in deficient cardiac filling. This conclusion agrees with that of our previous paper" (Conclusion 3) (4).

If both the heart and the peripheral circulation are, as is well known, affected by the action of digitalis, the problem in the case of normal hearts is this, how far is the effect of digitalis on the volume output exercised on the heart itself and how far on the amount of the venous return?

That there is a difference in behavior between enlarged hearts and hearts of normal size, and that this difference is of deciding importance, will be shown in these studies. What the relative share is of heart and periphery will also be discussed.

There is already evidence of the course of events in heart-lung preparations where a digitalis body is given to the heart in an enfeebled state. Hearts that were enlarged or failing have been studied by Anitschkow and Trendelenburg (5) who showed that by increasing the inflow (raising the venous reservoir in heart lung preparations), the output, after the heart was made to fail was much greater than before if strophanthin were injected, and that corresponding to this effect the pressure in the right auricle fell. Bijlsma, and Roessingh (6) noticed that in heart-lung preparations, when the heart was in process of dilating, it became smaller after giving strophanthin, though the resistance, the arterial pressure and the volume output remained unchanged. Plant (7) also has shown that when strophanthin is given to a failing heart, poisoned previously during two to three weeks with phosphorus so that it is the subject of fatty degeneration, the volume output increased, the pressure in the inferior vena cava decreased, and the amplitude of contraction increased as did the blood pressure.

Here then are three sets of experiments, based on the study of failing hearts, all enlarged, in heart lung preparations, in which increase in output went parallel with fall in venous pressure (Anitschkow and Trendelenburg, and Plant) and in which decrease in the size of the heart took place (Bijlsma and Roessingh). The effects were transient (Plant), the initial situation having been restored in 20 minutes.

The phenomena which are now reported were observed in hearts of heart-lung preparations, during a period of several hours until the time when complete failure of the preparation seemed imminent. As is well known, such preparations permit the control and measurement of several functions of which the circulation depends—the venous pressure, the volume output, the arterial resistance, the temperature. Obviously, hearts in heart-lung preparations cannot be regarded as fresh, or normal, or natural, due to the unwonted manipulations to which they have been exposed and also to the fact that they have been deprived of some of their extrinsic nerves. We do not, as a matter of fact, so regard them. Evidence that such hearts should not be considered fresh may be inferred from the fact that under the influence of digitalis, the minute output, unlike its effect in natural preparations and without regard to what ultimately will be held to be the mechanism, has not been seen to decrease, irrespective of the volume of the inflow.

For these experiments dogs weighing from 10 to 15 kilograms were used. Heart-lung preparations were made according to the general technique of Knowlton and Starling (8). The arrangements were varied, as will be pointed out, to suit the purposes of these experiments. Chloralose in physiological salt solution, neutralized by the addition of sodium hydroxide, served as anesthetic, and was injected intravenously usually in amounts of 0.1 gram per kilogram of body weight, but sometimes of 0.2 gram and rarely an addition of 0.5 gram. No morphine was given.

Blood for the experiment was secured from a second dog. Heparin, 5.0 mgm. diluted in 100 cc. normal salt solution was injected first into a femoral vein, then, under local, novocaine, anesthesia, the blood required was withdrawn from the carotid artery. To complete the process of bleeding, light ether anesthesia was administered.

The dog lay in the lower half of a box. On opening the chest, ventilation was maintained by a Starling pump. When the preparation was complete, a lid closed the box which was then made airtight. Air in an amount sufficient to create the desired level of negative pressure was then removed by a large piston pump. To vary the degree of negative pressure and by so doing to secure motions of the lungs, air was allowed to enter the chamber from a 3 liter rubber bag (*RB*) through a valve (*V*, Fig. 1) the opening and shutting of which was managed automatically by the motions of the pump. The pressure in the box varied between  $-2$  cm. and  $-4$  cm. water during expiration (shutting of the piston) and between  $-12$  cm. and  $-15$  cm. during inspiration. In normal animals the range in the intact pleural cavity is between  $-2$  cm. and  $-12$  cm. The mechanism of the box was designed to simulate that of respiration in the normal chest.

Certain details in technique are described

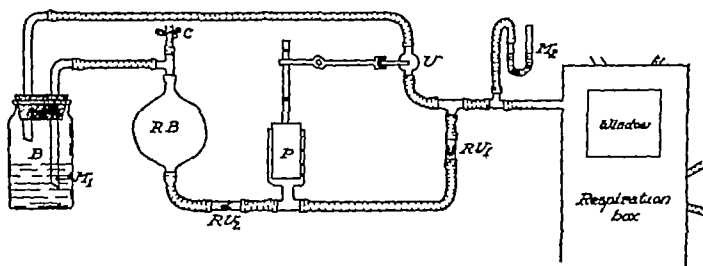


FIG 1 THIS DIAGRAM SHOWS THE ARRANGEMENT OF THE SYSTEM USED IN ARTIFICIAL RESPIRATION IN THE HEART LUNG PREPARATION  
The details are described in the text.

1 An upstroke in the pump ( $P$ ) (capacity 4 liters) drew air from the box through the valve ( $RV_1$ ), excluding at the same time, by closing the valve ( $V$ ), that part of the circuit between valve ( $V$ ) and valve ( $RV_2$ ). A downstroke of the pump sent air through valve ( $RV_2$ ), a rubber bag ( $RB$ ), a water manometer ( $M_1$ ) and so back to the box through valve ( $V$ ), which had meanwhile been opened by the downstroke of the pump. A leak in the system was detected by ballooning of the bag ( $RB$ ), distension of which could be relieved by opening a cock ( $C$ ). The pressure in the system could be modified by the level of water in the bottle ( $B$ ). A manometer ( $M_2$ ) indicated changes in pressure in the box. By bubbling through water (in bottle  $B$ ), the air in the system was kept moist.

2 When changes in the size of the heart were measured it was enclosed in a glass oncometer (Henderson) supplied with a rubber diaphragm, in which a hole was made, fitted snugly to the auriculoventricular groove (Figure 2). The oncometer was connected by pressure tubing with a volume recorder. At first a piston recorder (Huerthle) was used, next a cylindrical spirometer and finally, and also most satisfactorily, a Gad spirometer. The record of changes was inscribed on smoked paper.

3 The mean blood pressure in the brachiocephalic artery was recorded on the same smoked paper by a mercury manometer, that in the right auricle by a water manometer.

4 Peripheral resistance was provided in the usual manner (Knowlton and Starling (8)). Two resistance sleeves were placed in parallel in case of accident. The constancy of this system was checked by frequent readings of a mercury manometer.

5 The volume output was measured in a graduated cylinder, 50 ccm or 100 ccm being allowed to flow in on turning a three way stopcock, the time required being taken by a stop watch. Since the stop watch was in an electric circuit the duration of the period was recorded on the smoked paper.

6 The height of the head of pressure of the blood flowing into the heart was kept constant. The reservoir was a flexible rubber bag immersed in a large jar of water (9). Through a tube of large bore (1") water in the beaker was displaced and passed freely to a bottle suspended by rubber when blood in the reservoir exceeded a previous volume, conversely, decrease in the volume of blood in the reservoir invited a return flow of water into the beaker. The level of fluid once set remained, therefore, constant. By a rack and pinion, the height of this unit was easily adjusted.

7 A stopcock, with large bore, was placed between the reservoir and the right auricle. A dial showing divisions mounted on the housing, and a pointer sealed to the cock, permitted the repetition of a given setting. The inflow could accordingly be reduced, simulating constriction of the veins.

8 A thermometer, placed in the cannula inserted in the superior vena cava, indicated the temperature of the blood (not shown in the figure).

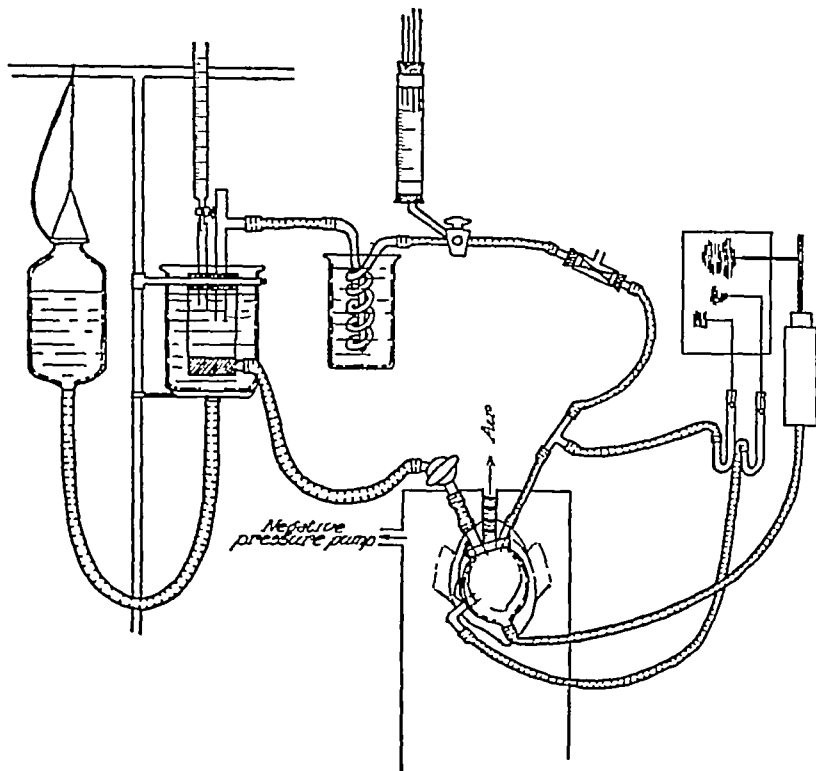


FIG 2 THIS DIAGRAM SHOWS THE ARRANGEMENT OF THE SYSTEM USED FOR THE CIRCULATION OF BLOOD IN THE HEART-LUNG PREPARATION

9 A solution of glucose and insulin flowed continuously into the venous reservoir from a graduated burette.

10 From a pet-cock, placed in the circuit just before the venous reservoir, blood was taken for analysis, and substances, such as digitalis (Digitan, Merck) were injected (not shown in the figure).

11 Time was recorded on the smoked paper at intervals of 1 second and 10 seconds.

#### PROCEDURE

Experiments of three types were performed. In the *first*, the ventilation was managed by the method of negative pressure—and without an oncometer about the heart. Although the degree of negative pressure which was exerted was comparable to that in the normal chest, when the bony thorax was divided and the edges were held apart, the lungs fell away from the heart and failed, therefore, to give it that support which normally protects it from the full force of negative pressure. How great

this effect may be is observed in the record in which fluctuations due to this influence are clearly observed in the records of arterial and venous pressures. The latter ranged between  $-3$  cm and  $+1$  cm water. The *second* type of experiment was like the first except that an oncometer was placed over the heart. The result of this arrangement seemed to approach more closely that in a normal intact chest. The venous pressure ranged between  $+2$  cm and  $+5$  cm water. In this type is included a sub-group (5 experiments) in which was studied the effect of varying the volume of inflowing blood. In a *third* type, ventilation was positive, the Starling pump being employed. An oncometer was again fitted over the heart. This arrangement exhibited the least effect of respiration on the arterial and venous pressures and on the size of the heart. The venous pressures were the highest observed, ranging from  $+5$  cm to  $+8$  cm of water. They were lowest in the first group.

The arrangements employed, taken together, provided a range of venous pressures running from a level above to one below normal. Though the natural situation was absent, it has been possible, nevertheless, to test the action of digitalis under very varying conditions, within the range of which, many, even the normal ones must have been present. Though different in degree, the effects of giving digitalis tended, whatever the arrangements, in the same direction.

The results in each type of experiment are illustrated by describing one example in detail.

When the heart was not enclosed in an oncometer, it was the usual experience (Figure 3) to observe on transition to negative pressure that,

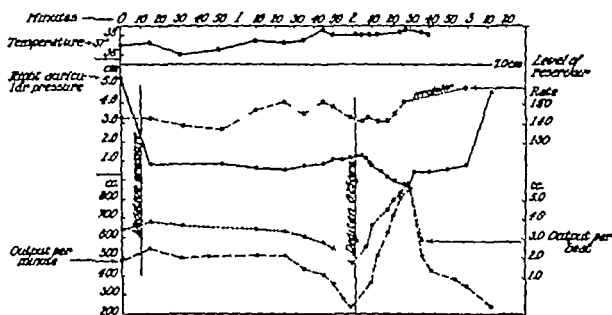


FIG 3 THE CURVES ARE SHOWN OF THE COURSE OF EVENTS IN EXPERIMENT NUMBER 44

The details are given in Table 1

due no doubt to the pull exerted on the heart, for this effect did not appear when it was protected by an oncometer, pressure in the right auricle



TABLE 1

March 5, 1931 Dog number 44 Weight of dog—10 kgm Weight of heart—88 grams  
Anesthesia—Chloralose—1.2 gram

Time	Tem- pera- ture	Arterial press- ure	Right auricular pressure	Rate	Volume in reser- voir	Output per minute	Output per beat	Changes in heart volume	Glucose burette level	Blood sugar
<i>a m</i>	<i>° C</i>	<i>mm</i> <i>Hg</i>	<i>cm</i> <i>H<sub>2</sub>O</i>	<i>per</i> <i>minute</i>	<i>cc</i>	<i>cc</i>	<i>cc</i>	<i>cc.</i>	<i>cc</i>	<i>mgm per</i> <i>100 cc</i>
10 52	Operation began									
11 30	Circulation turned over Level of venous reservoir set at 7.0 cm									
<i>p m</i>										
12 00	37.0	100	+5.3	142	625	480	3.4		1.1	93
12 10	Changed to negative pressure respiration									
12 15	37.2	106	+0.8	142		540	3.8			
12 31	36.0			138		490	3.6			
12 58									5.8	111
1 25	37.2	108	+0.5	150		500	3.3			
1 45	38.5	110	+0.8	150	525	400	2.7			
1 50	38.0	110	+1.0	148		350	2.4			
1 55			+1.0			270				
1 59		106	+1.1	142		230	1.6		24.4	172
2 03	Digitan		+1.2							
	0.25 gram									
2 06	38.0	106	+1.1	142	500		2.5			
2 09	38.0		+0.85	142		360				
2 10			+0.7				3.6			
2 14	38.0		+0.4	140		500				
2 18			+0.1	140		620	4.4			
2 22			-0.1	144		710	4.9			
2 25	38.2	110				800	5.3			
2 27	38.5		-0.3	150	490	850	5.4		34.3	202
2 36	38.2			Irregular		500				

fell abruptly. At the same time the output of blood per minute and per beat from the heart increased slightly. Under negative pressure, the pressure in the right auricle remained practically stationary for about one hour. For about another half-hour it fell slightly, while the volume output decreased, also slightly. Then the heart began to fail, as is shown by the rise in pressure in the right auricle and in the sharp fall in volume output. The temperatures, meanwhile, underwent no important change. The arrangement of the "venous" reservoir remained constant throughout the experiment, the height being 7.0 cm, it seems doubtful whether the changes in the rate of the heart were influenced by the alterations in temperature which occurred. The heart was permitted to fail until, after two hours, the output per minute fell to about 225 ccm. Synchronously, the pressure in the right auricle rose slightly, but distinctly. Digitalis (in the form of digitan, 0.25 gram<sup>3</sup>) was then injected. One

<sup>3</sup> This dose was calculated on the basis of 0.023 gram per kilogram. An amount having been calculated, as being suitable for intact animals, a smaller dose was given, taking the nature of the preparation into consideration. Later, doses still smaller were given.

half-hour after injection, the output increased as much almost as four hundred per cent, and was greater by about fifty per cent (550 ccm to 825 ccm) than the output just after the preparation was placed under negative pressure. During this period the pressure in the right auricle decreased to a point lower than during the early, steady period, falling so low indeed, that negative pressure was recorded, the ventricles were ejecting blood at least as fast as it was being supplied. An excess in the reservoir prevented exhaustion of the supply.

Too much digitalis, obviously, was injected, for the rate of the heart, having been steady for almost twenty minutes, increased rapidly, the rhythm then became quite irregular. The heart was irretrievably intoxicated. The output fell rapidly and in forty minutes was as small as just before digitalis was given. Toward the end of the experiment, pressure in the right auricle rose.

The course of events in this experiment is clear enough. At first the preparation was sufficient. When it was near the point of death, the administration of digitalis revived it. Its effectiveness then was greater than it had been throughout the period of observation. Clear as is the demonstration that a dying heart can be revived, it was impossible to estimate what changes took place in its size, nor was it clear whether the heart was enlarged when digitalis was injected.

The next experiment (the *second type*) gives information on this point. The heart was enclosed in an oncometer. And it gives information also on another point. The stopcock, already described, was inserted in the tube leading to the right auricle. When the box in which the dog lay was subjected to negative pressure, that fall in pressure in the right auricle which took place when the heart lay bare, now, when an oncometer was fitted to it, failed to occur.

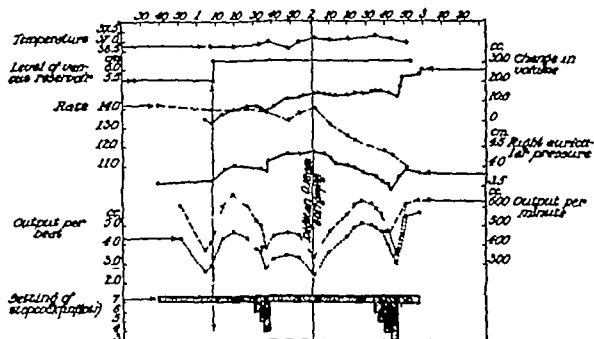


FIG 4 THE CURVES ARE SHOWN OF THE COURSE OF EVENTS IN EXPERIMENT NUMBER 59

The details are given in Table 2

TABLE 2

April 20, 1931 Dog number 59 Weight of dog—10.5 kgm Weight of heart—90 grams  
Anesthesia—Chloralose—1.15 gram

Time	Temperature	Arterial pressure	Right auricular pressure	Rate	Volume in reservoir	Inflow setting	Output per minute	Output per beat	Changes in heart volume	Glucose burette level	Blood sugar
<i>a m</i>	$^{\circ}\text{C}$	<i>mm Hg</i>	<i>cm H<sub>2</sub>O</i>	<i>per minute</i>	<i>cc</i>		<i>cc</i>	<i>cc</i>	<i>cc</i>	<i>cc</i>	<i>mgm per 100 cc</i>
11 18	Operation began										
11 58	Circulation turned over Level of venous reservoir set at 5.5 cm and changed at 1.12 to 6.5 cm										
<i>p m</i>											
12 50		102	3.5	142	400	7	600	4.3	0	7.9	112
1 06	36.8	108	3.6	140			410	2.9	-1.5		
1 12		108	3.9				600	4.3	+3.0		
1 18	36.8	110	4.0	140	400		660	4.7	+5.5		
1 25				140			600	4.3	+5.0		
1 28						6					
1 30	36.9						520	3.7	+7.0		
1 31						5					
1 32			3.9				500	3.6	+6.0		
1 33						4					
1 35			3.85	138			380	2.8	+4.5		
1 35+						7					
1 36	37.0		4.1				440	3.2	+7.0		
1 46	36.7	114	4.3	134			460	3.4	+11.0		
1 52	37.0		4.3	138			450	3.3	+11.5	19.6	141
2 00	37.2	114	4.35	140			330	2.4	+13.5		
2 01	Digitan 0.18 gram										
2 08	37.1		4.2	132	375		475	3.6	+13.0		
2 17	37.2	114	4.0	126			560	4.4	+13.0		
2 21		114	3.95	124			610	4.9	+13.5		
2 25		114	3.85				630	5.1	+14.0		
2 30							610	5.0	+14.5		
2 31						6					
2 32	37.3		3.70				590	4.9	+15.0		
2 33						5					
2 35			3.60				560	4.7	+15.0		
2 36				118		4					
2 39			3.50				460	4.0	+14.0		
2 40						3					
2 43	37.2		3.40	116	125		350	3.0	+12.5		
2 44			3.7			7			+17.5		
2 46					325 (200 cc blood returned)						
2 48	37.0	116	3.9	110			610	5.7	+22.0	29.0	174
2 55		116	3.8	110			630	5.7	+24.0		
2 56	Rubber for peripheral resistance broke										

After about thirty minutes (Figure 4), the level of the venous reservoir having been set at a height of 5.5 cm, the volume output per minute, then about 600 ccm, fell in the next ten minutes to 380 ccm. The level of the reservoir was then raised to 7.0 cm, where it remained throughout the experiment. The output rose to about 630 ccm, higher than it had been

before The volume of the heart increased, the pressure in the right auricle rose The behavior of the heart having been satisfactory for fifteen minutes, the stopcock in the inflow tube was closed bit by bit Output per minute and per beat, pressure in the right auricle and the volume of the heart, all promptly and expectedly decreased, and decreased the more, the more nearly the stopcock was shut After ten minutes the stopcock was again opened wide At once, output, volume of the heart and pressure rose, but not to the initial height. The heart seemed no doubt to have been deteriorating, as is shown by its increasing volume After another twenty minutes the preparation began seriously to fail, the output decreased to about 325 ccm, the pressure in the right auricle rose and the cardiac volume increased still further The temperature of the blood and the rate of the heart beat remained meanwhile sufficiently steady Digitalis was now injected In ten minutes a substantial increase in volume output occurred and in thirty minutes the amount put out at one hour had almost been reached The volume of the heart, which before injection had steadily been increasing, now ceased to do so, on the contrary, it became a little smaller The pressure in the right auricle promptly declined, falling to a level lower than when the output was approximately the same as now As the rate of the heart beat fell, the output per beat increased, so that the curve describing this function continued roughly parallel to that of the output per minute There was, nevertheless, no increase in the heart's size Half an hour after the injection of digitalis, the maneuver with the stopcock was repeated The course of events noticed before took place again The minute output and output per beat declined, the pressure fell, the heart's volume decreased When the stopcock had been shut to a point identical (marked by asterisks on the stopcock and also on "output per minute" curves) with that before injection, it is apparent that, although the minute output just before closing the stopcock was approximately alike on both occasions, now, after the injection of digitalis, the same setting resulted in a smaller effect than before—the minute output did not decrease so much It was greater in fact by about 80 ccm, an amount equal to 12.0 per cent of the highest output. A tighter shutting of the stopcock had, of course, a correspondingly greater effect, as the dotted line at 2.25 shows Together with the change in minute output, corresponding changes in the volume of the heart and in right auricular pressure took place. The rate of the heart beat continued to fall so that the curve describing output per beat is higher than before When the stopcock was opened again, the output increased to its former amount, the pressure in the right auricle also returned, but showed no disposition to rise further, evidence of the good state of the preparation Shutting the stopcock seems, however, further to have damaged the heart for now in order to maintain its output, it dilated sharply

This experiment shows that (1) Giving digitalis when the heart was in a state of acute failure, revived it, so that the initial minute output was restored (2) Although before the administration of digitalis the heart was continuously dilating, afterward, further deterioration was arrested, until decreasing the inflow again, by shutting the stopcock, still further injured the heart (3) Decreasing the inflow by means of the stopcock exerted a smaller effect on the minute output, after giving digitalis than it had before Exactly how this difference is to be "explained" is not apparent Greater contractile efficiency, decrease in rate, balanced by greater output per beat, made it possible to receive and to expel greater volumes of blood, the reservoir arrangements having remained unchanged That the volume of flow through the heart was greater is shown by the fall of pressure in the right auricle (4) The effect just described is characteristic of this preparation, free as it is of hepatic and all other veins save the pulmonary The failing heart, it appears, is able, despite the decrease in inflow brought about by constriction of the veins, simulated by shutting the stopcock in this case as much as about 40 per cent, to expel more blood at the same setting under the influence of digitalis than before its administration Inflow is in short a function not solely of the degree of constriction of the inflow tract, the state of efficiency of the heart, its ability to receive and to eject blood, is another relevant factor

Still other experiments (the *third type*) were performed in dogs in which respiration was maintained by positive pressure, a Y-shaped cannula being fixed in the trachea, the two limbs of which were connected with the inlet and outlet tubes of a Starling pump, and in which an oncometer was fitted over the heart In an illustrative experiment (Figure 5) failure of the heart was signalled by a fall in volume output from 700

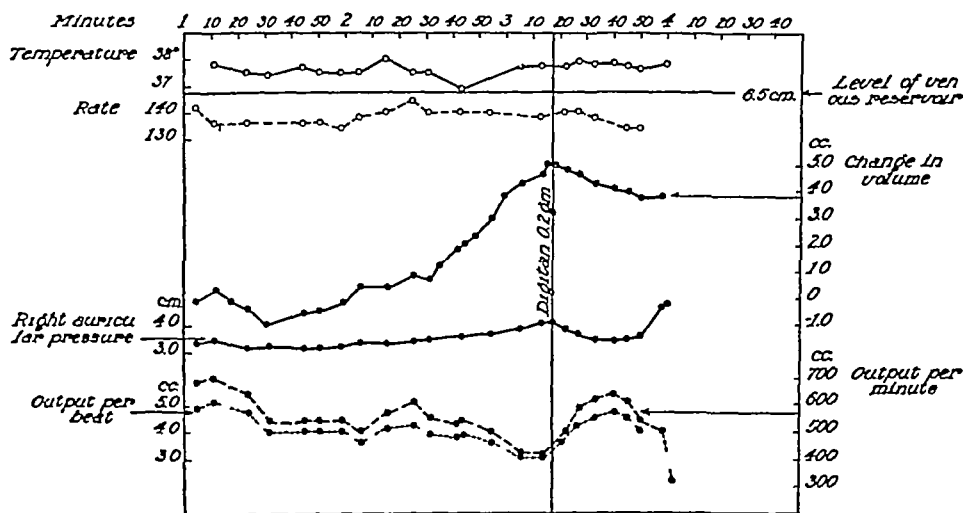


FIG 5 THE CURVES ARE SHOWN OF THE COURSE OF EVENTS IN EXPERIMENT NUMBER 65

The details are given in Table 3

TABLE 3

June 10, 1931 Dog number 65 Weight of dog—115 kgm Weight of heart—109.5 grams Anesthesia—Chloralose—1.25 gram

Time	Temperature	Arterial pressure	Right auricular pressure	Rate	Volume in reservoir	Output per minute	Output per beat	Changes in heart volume	Glucose burette level	Blood sugar
a.m.	C.	mm Hg	cm H <sub>2</sub> O	per minute	cc.	cc.	cc.	cc.	cc	mgm. per 100 cc.
11.28	Operation began									
12.21	Circulation turned over Level of venous reservoir set at 6.5 cm									
1.04		106	3.4	142	330	690	4.9	(100 cc. blood added to reservoir)		
1.07					620					
1.11	37.8	106	3.5	136		700	5.1	+0.4	0.1	82
1.23	37.5	104	3.2	136	700	640	4.7	0		
1.31	37.4	104	3.25			540	4.0	-0.9		
1.44	37.7	104	3.2	136	600	540	4.0	-0.5		
1.50	37.5	104	3.2	136		540	4.0	-0.5		
1.58	37.5	104	3.25	134		540	4.0	-0.1		
2.05	37.5	104	3.4	138		500	3.6	+0.5	38.5	402
2.15	38.0	106	3.35	140		570	4.1	+0.5	(Blood noted in pericardium)	
2.25	37.5	106	3.4	144		610	4.2	+0.9		
2.31	37.5	106	3.5	140		550	3.9	+0.8		
2.41						530	3.8	+1.8		
2.43	36.9	106	3.6	140		540	3.8	+2.1		
2.54		106	3.7	140		500	3.6	+3.1		
3.05	37.7	106	3.9			420	3.0	+4.4		
3.13	37.7	105	4.1	138	200	410	3.0	+5.1		
3.17	Digitalin 0.2 gram		4.15						48.8	402
3.22	37.7	106	3.9	140		500	3.6	+4.9		
3.27	37.9	106	3.7	140		590	4.2	+4.7		
3.33	37.8	106	3.5	138		620	4.5	+4.4		
3.40	37.8	106	3.45			640	4.7	+4.2		
3.45	37.7	106	3.5	134		610	4.5	+4.1		
3.50	37.6	106	3.6	134		540	4.0	+3.9	52.3	436
3.58			4.7			500	?			
4.00	37.8		4.8			310				

ccm to 410 ccm per minute. The heart dilated, the venous pressure rose. Important changes did not take place in the pulse rate or in the temperature. When digitalis was injected, the minute output rose to almost its original level. There was prompt and striking decrease in its volume, the decrease continuing for a short time after the maximum output was attained. In all probability, the pericardium being open, the heart even then was relatively dilated, in comparison with its original, natural size. The change illustrates the fact, however, that, when heart muscle fibers are stretched more than a certain length, shortening does not, as is the case of normal hearts, decrease but rather increases the minute output. Coincident with the rise in output, occurred a distinct

fall in right auricular pressure The rate of the heart beat also fell but only six a minute

Propelling blood against more than an atmosphere of intrapulmonary pressure did not in this experiment interfere with the ability of the heart to increase its output when digitalis was administered It is striking, furthermore, how great were the concomitant effects on the size of the beat and on the pressure in the right auricle Despite decrease in volume, the output was increased to so great an extent that it was able to receive blood and to propel it onward toward the lungs so competently as to lower the pressure in the right auricle very considerably

Twenty-three experiments in all are reported In one-half the final effect of the action of digitalis was to decrease the rate of the heart, though in one-half of these, the fall was preceded by a slight rise In two experiments only did the rate increase promptly and permanently In the remainder it remained practically constant These statements refer only to rates during periods of normal cardiac mechanism As terminal phenomena, paroxysms of ventricular tachycardia were frequently observed, and on a few occasions recoveries from paroxysms, in early stages In no instance did the rate of the heart beat change sufficiently so that alterations in it could be regarded seriously as responsible, during the period of the action of digitalis, for altering the cardiac minute output

Five experiments were valueless for the purpose of securing measurements of venous pressure, of cardiac output and of changes in size of the heart In one case (Number 63) clots formed in the circulating blood, in two others (Numbers 52 and 60) large hemorrhages took place in the cardiometer, and in two additional ones (Numbers 55 and 57) ventilation under negative pressure failed The two cases last mentioned are, however, not without interest Although they cannot yield measurements comparable with those found in other instances, they do, nevertheless, suggest reasons which explain the failure of the action of digitalis (Table 4)

The remaining eighteen experiments (Table 4) were sufficiently satisfactory to permit deductions to be made from the measurements In all of them, the temperatures and the rate of the heart beat were approximately constant, these factors need, therefore, not to be taken further into consideration The venous reservoir remained at a constant level after it was definitely set Since the pressure level of the "peripheral resistance" varied within slight limits only during the course of an experiment, changes in brachiocephalic blood pressure did not occur For these reasons, the following six variable factors only require study (1) the minute output from the heart, (2) the size of the heart, (3) the pressure in the right auricle, (4) the condition of the heart, (5) the condition of the lungs, and finally (6) the condition of the blood which is in turn dependent on the state of the lungs and the rate and volume of the blood

flow Since experiments showing gross changes in the lungs and in the blood were not further studied, the last two factors (5) and (6) may be ignored There remain, therefore, only four variable factors The first three can all be measured and since in fact they are dependent on the condition of the heart, measurement of them describes in turn the state of this organ Changes in these measurements depend, therefore, upon changes in the heart It should be possible, consequently, by comparing measurements made before giving digitalis with others made afterwards, to describe certain of the effects for which the administration of this drug is responsible (Table 4) Descriptions of three typical courses of events have been given already The following description may serve, therefore, as a general summary

When the pressure in the right auricle rose slowly and steadily, the volume output per minute and per beat decreased, and the size of the heart simultaneously increased A cardiometer was employed in ten only of the experiments In one case (Number 60) a leak of air invalidated the results But in the other nine instances, measurements made it certain that the hearts dilated In the eight cases in which an oncometer was not used, it seems certain also that the hearts increased in size

The effect of the administration of digitalis was prompt and definite as measured by the three criteria on which reliance was placed the minute output, having fallen, rose, the pressure in the right auricle, having risen, fell, the heart, having dilated, contracted These results were noticed in fourteen of the eighteen satisfactory experiments Of the remaining four, one (Number 57) only showed no effect, but in the other three (Numbers 49, 52, 55) the effects were either slight or their progress having begun was promptly halted so far as concerns reduction in size of the heart and rise in right auricular pressure, though in all three the output exhibited a slight rise

#### DISCUSSION

These experiments, it seems, supply evidence which shows that hearts which are obviously failing, in that, although constantly increasing in size they are expelling ever smaller volumes of blood can, nevertheless, as a consequence of receiving digitalis, deliver greatly increased quantities Since there are no blood vessels, except the bronchial and pulmonary vessels, nor any other structures in the preparation than the lungs, no other mechanism can be responsible for this occurrence than the heart itself The heart can apparently become smaller and at the same time more efficient in increasing the volume of blood which it expels That an effect of the action of digitalis is exercised on heart muscle is well known, and also that this effect may consist at one stage in increasing the degree of its systolic contraction Experiments have shown in fact that "improvement" in the behavior of the heart takes place also in damaged and



TABLE 4

*A summary of the individual experiments in the three groups*

Dog number	Change of temperature	Rate	Right auricular pressure	Volume output per minute	Volume output per beat	Change in volume of heart	Duration after digitalis	Dose	Constriction of inflow
	° C	per minute	cm H <sub>2</sub> O	cc.	cc.	cc	minutes	grams	
Group I Negative pressure, no oncometer									
36	0	128 † 134	+ 0.4 - 3.0	290 600	2.4 3.4		30	0.5	
37	0	138 142	- 1.2 - 4.0	200 470	1.4 3.4		20	0.3	
38	0	138 114	+ -	90 240	+		40 10	0.3 0.1	
39	0	146 126	+ 2.2 - 1.0	240 920	+		30	0.3	
41	0	144 114	+ 0.8 - 2.0	200 510	+		55	0.25	
42	0	118 110	- 3.6 - 4.6	200 420	+		65	0.25	
44	0	140 150	+ 2.4 - 0.8	230 850	1.6 5.7		70	0.25	
45	0	166 186	- ?*	610 760**	3.6 4.1		100	0.25	
Group II Negative pressure, with oncometer									
49	+1.2	134 138	+20 +20	- ?*	- ?*	†	37	0.25	
50	-1	140 168	+ 4.4 + 2.2	690 1070	4.6 6.1	-1	44	0.25	
51	0	154 156	+ 7.4 + 6.0	600 700**	+ ?	-8	35	0.25	
52	0	136 136	+ 5.2 + 5.2	- ?*	?	?	35	0.25	

† Whenever two figures appear in a space the upper represents the value recorded immediately before the administration of digitalis, the lower the maximum effect of the drug

\* Continual decrease during the experiment

\*\* Continual increase during the experiment.

† Oncometer leaked

TABLE 4 (continued)

Dog number	Change of temperature	Rate	Right auricular pressure	Volume output per minute	Volume output per beat	Change in volume of heart	Duration after digitan	Dose	Constriction of inflow
53	C 0	per minute 136 128	cm. H <sub>2</sub> O + 7.8 + 7.2	cc. 310 500	cc.  +	cc §	min 24	grams 0.20	
54	-0.7	120  116	+ 8.9  + 9.0	290  470	2.6  4.4	§	87	0.15	- 80 cc - 19.5 per cent  No reduction
55	+1.7	126 112	+ 4.7 + 4.8	260 270	?  ?	+?	36 5	1.5 0.1	
56	0	140  104	+11  +10.6	300  580	  +	  -5	73	0.2	-270 cc - 45 per cent  -210 cc. - 36 per cent
57	0	134 134	+ 4.0 + 4.0	340 300*	-?  -	-***	35	0.18	Reduced Not done
59	0	140  110	+ 8.7  + 7.7	330  630	2.4  5.1	  -1½	56	0.18	-220 cc - 36.7 per cent  -150 cc. - 26.0 per cent
60	0	134 132	+ ?**	360 300*	-?  -	-?†	25	0.18	Reduced Not done

## Group III Positive pressure with oncometer

63	-1.5	134 130	+ 9.0 + 8.0	320 370	+?  +	†	34	0.18	
64	0	148 150	+ 9.8 + 9.0	390 460	+  +	-6.0*	48	0.2	
65	0	138 134	+ 8.3 + 6.9	410 640	+  +	-1.2	45	0.2	
66	0	128 120	+14.2 +11.7	300 590	+  +	-3.1	63	0.2	

§ Further increase in size postponed for varying periods of time although no decrease occurred

\*\*\* After revival by positive pressure

feeble hearts But these experiments supply evidence not only that damaged and feeble hearts can be so influenced but also that hearts which are dilated can And they show, furthermore, that when they expel greater volumes, they may at the same time diminish in size Smaller hearts manage, in short, to increase the volume output

This situation is different from that in hearts which, because obviously undamaged, are of normal size and in certain other hearts which are damaged but are not dilated, even as the result of forced exercise (Harrison and Leonard (1), Cohn and Stewart(2)) The hearts which were studied in the experiments now reported were plainly not fresh hearts To draw inferences from their use, as if they were, would be unjustified But the point at issue has not to do with whether they were fresh or old, but essentially with whether the effect of digitalis is exerted in a significant fashion on the heart muscle, and with what that effect is If its action on enlarged hearts, dilated or hypertrophied, is admitted, but an effect on normal sized hearts is denied, a point in the process of lengthening must be found at which the muscle *begins* to be susceptible to the action of the drug There may be such a point, but its presence has not, so far, been the subject of investigation The volume outflow may under definite circumstances reflect the action of other influences But to arrive at a complete description, it is not enough to study only the peripheral action of digitalis Its cardiac action has consequently been investigated in detail In other experiments, to attempt to ascertain what effect on the net result might be contributed by an action on the peripheral circulation, especially in the portal and hepatic regions, the stopcock was placed in the inflow tract to the heart Reference will be made to this type of experiment later

The decrease in volume output which has been noticed was thought to result from the tonic effect of digitalis upon the heart (2), in consequence of which its size diminished so much, that a large or even a usual volume of blood could not be received and, therefore, could not be ejected Dock and Tainter (3, 4) believe that an explanation of this sort must be rejected because it fails to take into account the behavior of the rest of the circulatory apparatus which, as they think, is simultaneously affected They have drawn attention (4) to a mechanism in the hepatic veins by which, under the influence of digitalis, the lumina, the walls containing smooth muscle which contracts, diminish and prevent the return of blood to the heart (10) because it is dammed back into the intestinal cavity The decrease in output from the heart results accordingly not from a primary effect upon the dimensions of the heart but from a secondary one due to constriction of the hepatic veins The heart becomes small, not because of the effect upon it of the action of digitalis but because it can not be filled

Now the vessels which transfer blood from the descending aorta to

the inferior vena cava may be said to consist of a main circuit, returning blood chiefly from the kidneys, the pelvis and the legs, and a shunt which conveys blood to the intestinal viscera, chiefly to the spleen, the pancreas and the intestines, and which then passes through the liver, entering the inferior vena cava finally through the hepatic veins. The blood flowing into the right auricle is derived both from the superior and the inferior vena cava and the vena azygos. The portion which the hepatic veins contribute to the inferior vena cava is, as Grab, Janssen and Rein have shown (11) approximately 50 to 60 per cent or about one quarter of the amount which flows to the right auricle. On occasion this amount may be augmented to about 80 per cent or about one third of the total.

It seems to be a fact that not only individuals, but species vary considerably in the amount of smooth muscle which the walls of the hepatic veins contain. On this, when present, digitalis acts. In consequence of this action, the smooth muscle contracts and by so doing narrows the lumen so that blood is dammed back in the portal system. That this result occurs, especially in dogs, is known because the pressure in the portal system rises but the extent of the pooling, important in the mechanism only if great, is not known. But, as recent investigations have shown (12) it is dogs especially which exhibit this anatomical peculiarity. The hepatic veins of cats, for example, and of human beings fail to exhibit conspicuous amounts of the smooth muscle. Whatever may be the case regarding the mechanism in question in dogs, it cannot be operative in human beings because plainly it does not exist in them to a significant degree.

But in a given dog the possibility of the existence of this mechanism remains, capable of reducing the return flow to the heart to an extent so great that the total volume inflow and in consequence the size of the heart are reduced on this account to the extent that observation shows them to be.

The anatomy of the hepatic veins themselves requires, however, close scrutiny. They can, as has been said, be responsible for conveying 50 per cent of the blood which flows back into the inferior vena cava. They are in short each, right and left hepatic veins, large structures. Their united circumference exceeds that of the inferior vena cava (13). In order to be effective in shutting off from the volume of blood returning to the right auricle as much as a quarter, the lumina of the two vessels must be reduced to zero—must be completely obliterated. What the facts are is not known. The muscle in the walls of the hepatic veins, though abundant, seems scarcely capable of so powerful an action. But if they cannot close wholly they may conceivably close as much as 50 per cent. If they manage this, they deprive the inflow to the auricle of one-eighth (12 per cent) only of its total volume. And even if they succeed in bringing about this result, the diminution should be incapable of explaining

the facts found in earlier experiments (2a) The results that were found in those dogs were striking The outflow decreased not 12 per cent but in amounts varying between 34 and 68 per cent (with one exception in which it was only 14 per cent Table 5A) These are the consequences of giving

TABLE 5

*The effect of digitalis on the cardiac output in dogs with normal hearts (A) and with enlarged hearts, the result of artificial valvular lesions of long standing (B) \**

A		B	
<i>Dog number</i>	<i>Per cent decrease</i>	<i>Dog number</i>	<i>Per cent decrease</i>
265	66	162	58
257	38		67
258	48		32
253	46	161	62
254	45		31
252	35	158	75
251	53		65
255	34		47 4
264	14	155	44
259	50	171	83
261	68	90	23
263	47		

\* The data in this table are taken from the tables in papers by Cohn and Stewart (2a and b)

digitalis to dogs, the hearts of which were normal When similar experiments were attempted in the dogs in which the hearts were enlarged (hypertrophied) (2b) as the result of long standing damage to the mitral valves, the reduction in output was at least as great as in the normal ones and in some cases even greater (Table 5B) To refer the explanation of decreased outflow to decreased inflow—due to contraction of the hepatic and mesenteric veins seems, therefore, incorrect

But this explanation of the mechanism of diminished outflow seems unlikely for other reasons as well The effect of the action of digitalis upon the heart endures for days There are records (Cohn and Stewart) of the persistence of the action as long as 10 days, and durations of 3 days are not uncommon In human beings all the effects have often not disappeared even after 21 days In comparison with durations as long as these, those reported on the length of time the effect of the action of digitalis has been observed on the hepatic veins must be regarded as evanescent Unfortunately no example is shown (Tainter and Dock) in which the effect persisted longer than 25 minutes or, when digitalis and strophanthin were given in succession, for longer than 40 minutes The observations were then terminated If rise in pressure in the portal

system is taken as the duration of the action of these drugs on the hepatic veins, 5 to 12 or 15 minutes seems to be the maximum period. If the criterion is fall of pressure in the right auricle, this effect did not occur in Dog 1, began to disappear in Dog 2 after 14 minutes, and in Dog 4, after giving strophanthin, had regained its initial level in 20 minutes. It lasted somewhat longer, for 2 hours, in Dogs F4 and F5, when the records stop. The constrictor action of digitalis on the hepatic veins occurs, no doubt. When it fails to do so in Dog 1, the hepatic veins may be poor in muscle. Interesting though this action is, it seems to be transient. A point of importance should be made on the matter of the duration of effect. If, as between effect on heart and on hepatic veins, that on veins persists, it is essential to know in general how long this action may be. That on the heart is known to last for several, perhaps for many days. If that on the veins is as long, the action of both structures must contribute to the final result, if not, that on the veins possesses a slight influence only. The evidence concerning the effect on the veins so far assembled is meagre.

In intact dogs (2a and b) the effect of injection was studied 2 to 3 hours after injecting digitalis, except in two instances when the times given are  $1\frac{1}{2}$  to  $1\frac{3}{4}$  hours. And the persistence of the action is not minutes but 1 to 3 to 10 days. If, as seems to be the fact, the hepatic veins become narrow, the duration of this influence of digitalis is so brief that there must be hesitation in attributing to this effect of the drug, responsibility for the long persisting decrease in outflow, a decrease which does not begin to be effective, or at all events did not begin to be studied, until 2 hours (2a) after injection, and which persists for many days. The hepatic mechanism is plainly non-existent in any important sense. If objection is made to ignoring the short duration of the effect of digitalis on the heart in heart-lung experiments and to insisting upon it in connection with experiments on the hepatic and splanchnic veins, it may be pointed out that the two experiments differ. Those on the heart come to an end not because the action of digitalis ceases, but because it proceeds often to still greater and graver damage. The effect on the veins ceases because in all probability it is overshadowed by the results of trauma. Whether its action persists, nevertheless, is unknown. In intact animals the situation is different, the duration of the effect is longer but it has not been observed beyond two hours.

To test the relation of low venous pressure to decreased minute output, those experiments were performed in which the calibrated stopcock was inserted in the inflow tract. The pressure in the right auricle rose, as has been described, when the heart began to fail, it fell when the heart revived. There can be no question in these experiments of a venous (hepatic) mechanism, there were no veins. Nor can there have been any change in the volume of blood to which the heart had access, the amount,

due to the arrangement of the experiment was always the same<sup>4</sup> If a change in pressure took place, as it did, the behavior of the heart alone must have been responsible And this change, a fall in pressure in the right auricle, it was able to bring about even when, and this point is not without interest, a decrease in its size took place Decrease in the return flow is not the condition, obviously, which is responsible either for decrease in the size of the heart, for the outflow increased, or for the fall in pressure in the right auricle, because there was no decrease in the volume of blood ready to flow in

A further test could be made of the possible influence of constricted hepatic veins on the net result of giving digitalis, so far as output is concerned It has already been suggested that the maximum effect likely to take place as the result of constriction of the hepatic veins due to giving digitalis is a reduction in inflow of about 12 per cent If the veins were shut entirely the effect, an unlikely occurrence, would be 25 per cent When the heart in the experiments now reported began to fail, the cock to which reference has been made was partially shut, so that volumes less than the initial ones were available to the heart Reductions in caliber greater than 10 per cent effected a reduction in output but, and this is the point, the reduction in output at identical settings of the stopcock was greater in the undigitalized failing, than it was in the digitalized, heart Giving digitalis may constrict the hepatic veins so that the output from the heart is diminished but, as this experiment shows, this is a consequence depending not simply on the degree of constriction of the veins but on the difference in the heart after giving digitalis The dilated failing heart can, from the same reduced inflow path, manage a greater output, and in the intact animal when the entrance pathway is reduced to 75 to 87 per cent, the heart can still deliver a greater volume than before

But for the normal heart the restriction in inflow cannot, as has been shown, represent the facts Proof that this must be the mechanism on which decreased size of the heart and decreased output depend has been believed (4) to depend on the low level of venous pressure Evidence beyond that found in these experiments, which describe the course of events in failing hearts, to show that low venous pressure and small size together with diminished output, as is found in normal hearts, are not in necessary relation, is supplied in a study of the action of digitalis in normal human beings It is undesirable to anticipate the report (14) which describes such observations, but mention may be made now of the fact that, in them, measurement of the venous pressure fails to show, on the assumption which has been made, that this function of the circulation behaves in a way capable of explaining the facts

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<sup>4</sup> Those experiments in which the volume of the inflow is varied by changing the level of the reservoir are obviously different In them the head of pressure remains constant.

An effect on the tone of the heart appears to be, in the light of these considerations, the outstanding phenomenon. And by tone is meant the diastolic size of the heart, for this in a given case is indicative of the length of fiber, the length of fiber being, in turn, "synonymous with physiological condition or fitness of the muscle fibre" (Patterson, Piper and Starling (15)). This, as Bodo (16) remarks, was correlated by Starling and Visscher (17) with the oxygen consumption of the heart and the consumption of this "was determined by the initial length of its muscular fibres, i.e. by its diastolic volume." The heart's tone may be measured, therefore, in terms of its diastolic volume, "provided that the work performed by the heart is maintained constant during the whole of the experiment." Since, in the experiments of Cohn and Stewart the measurements were made during periods when the dogs were in so-called basal states, the work which their hearts performed may be regarded as constant, and the effects on diastolic size, as measures of the effect of the action of digitalis on tone. In the current experiments the situation is different. The amount of work which the hearts did, and the rate of the contractions varied, as well as the output per beat, although the resistance against which they worked was uniform. These were the effects which took place with deterioration of the preparation and they may be regarded as measures of the decrease in its effectiveness, expressed ultimately as increase in its diastolic size. If change in diastolic size is expressive of change in tone, change in tone took place in these experiments as well as in the ones formerly reported (2a, b) (Cohn and Stewart).

#### CONCLUSIONS

1 The minute output from failing, dilated hearts in dogs in heart lung preparations is increased when "therapeutic" doses of digitalis are administered. This effect is the opposite of that in the case of healthy hearts, normal in size.

2 When the output increases, the pressure in the right auricle decreases.

3 Increase in output is consistent with decrease in the diastolic volume of the heart.

4 If the inflow, and consequently the outflow, from the heart is restricted, the decrease in outflow is greater in the failing heart than in the same heart when it acts under the influence of digitalis. It appears from this test and from the discussion in the text that constriction of the hepatic veins is not a significant factor in the effect which the action of digitalis exerts on the size of the failing heart.

5 In estimating the value of a drug, its usefulness in therapeutics need not depend on its effect of any given function which presumably is correlated with the effect of that drug on the organism as a whole.





# STUDIES ON THE EFFECT OF THE ACTION OF DIGITALIS ON THE OUTPUT OF BLOOD FROM THE HEART

## II THE EFFECT ON THE OUTPUT OF THE HEARTS OF DOGS SUBJECT TO ARTIFICIAL AURICULAR FIBRILLATION

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That an effect of giving digitalis to normal unanesthetized dogs is to decrease cardiac output and size and to increase the extent of ventricular contractions has previously been reported (1) To study in dogs, phenomena comparable with heart failure with edema in human beings, experiments were undertaken employing several methods The one finally adopted was to bring about, artificially, insufficiencies of the mitral valve (2) After a lesion had been made by operation, the hearts increased in size The dogs exhibited no signs of heart failure, however and were able to run on a treadmill as normal dogs do When digitalis was given to them, the results were similar to those occurring in normal dogs These were decrease in cardiac output and cardiac size, and increase in the extent of ventricular excursions (2) A situation was, however, still wanting in which to test the effect of giving digitalis to animals the hearts of which were in a poor state functionally, a situation, in short, in which the output of blood per minute from the heart was diminished and the heart dilated Stewart and Gilchrist (3) and Stewart and Crawford (4) have shown that such a situation could be managed They found that when the hearts of normal dogs were made subject to auricular fibrillation, artificially induced, the volume output of blood per minute from the heart diminishes (3), and dilatation of the heart, detected by increase in size of its shadow in x ray photographs, may occur (4) These changes, decrease in output and increase in size of the heart, were already present one hour after the onset of auricular fibrillation Here then was a preparation suitable for testing in dogs the effect of giving digitalis when the functional capacity was diminished In this report there are described, accordingly observations on the effect of giving digitalis on the cardiac output per minute, cardiac size, ventricular and femoral pulse rates per minute in unanesthetized trained dogs during auricular fibrillation

## METHODS

All the dogs were trained to breathe in a Benedict-Roth spirometer for 20 to 30 minutes a day for ten days to two weeks before they were used. At the end of this time they lay quietly on the table without emotional disturbance, while measurements of oxygen consumption were made and other procedures were carried out. It was possible to decide at the first or second trial whether a given dog was a satisfactory subject. In the technique of measuring oxygen consumption, the rubber mask described by Blalock was used (5).

The operative procedure has already been described (6). Briefly, wire electrodes were sutured to the right auricle (Figure 1). The opera-

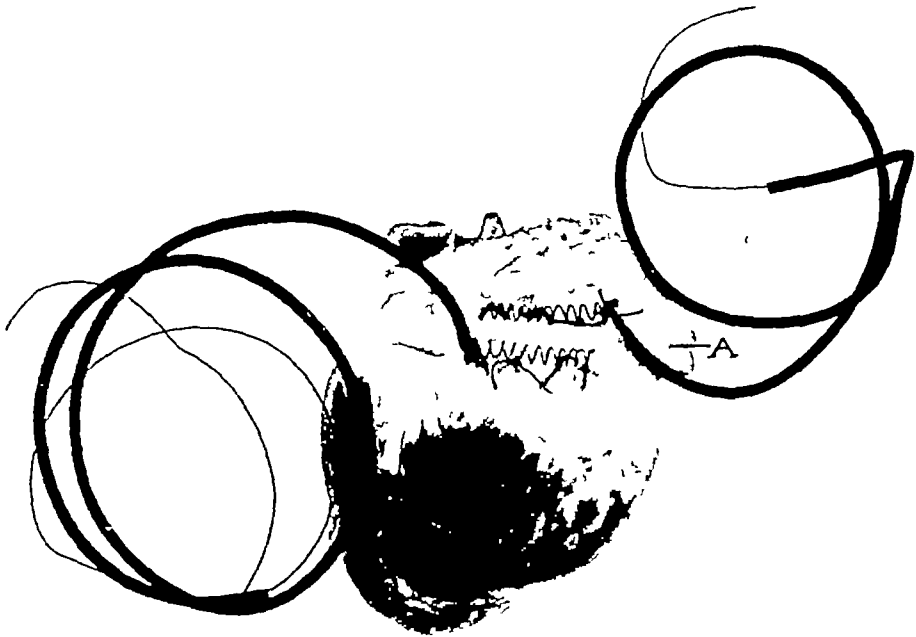


FIG 1 THIS FIGURE SHOWS THE MANNER IN WHICH THE SPIRAL WIRE ELECTRODES WERE SUTURED TO THE RIGHT AURICLE  
A is the right auricular appendix

tions were performed with sterile precautions, the dogs being anesthetized with ether given by the intratracheal method. After recovery from the preliminary operation, the auricles of the heart were made to fibrillate by stimulating them through these electrodes with faradic current obtained from one to three dry cell batteries placed in the primary circuit of a DuBois-Reymond induction coil. In order to be certain that the auricles were fibrillating, as well as to calculate the number of effective beats, a tracing of the femoral pulse was recorded simultaneously on the same film with an electrocardiogram (Figure 2)(7). The femoral pulse was

transmitted by rubber tubing from a rubber cuff applied to the right hind-leg through an Erlanger capsule modified by Kolls (8), and Kubie (9), to a Frank capsule placed in front of the camera. The rubber cuff on the leg was inflated with air to a pressure near the diastolic level. The movements of a beam of light focused on a mirror glued to the membrane of the Frank capsule were reflected to the lens of the camera and were traced on the moving sensitive film simultaneously with the electrocardiogram. Nonpolarizable electrodes were placed on the right fore-leg and the left hind leg for the derivation of Lead II of the electrocardiogram.

Samples of arterial blood were drawn from a femoral artery and those of mixed venous blood from the right ventricle by a special cannula inserted into that chamber through the right external jugular vein (10). The oxygen contents of these samples were estimated by the Van Slyke and Neill manometric method (11) for calculation of the arteriovenous oxygen differences. Immediately after the blood samples were drawn, the oxygen consumption was measured with a Benedict-Roth spirometer equipped with a graphic recording drum. Data were available, therefore, for calculating the minute volume output of the heart according to the Fick principle (12).

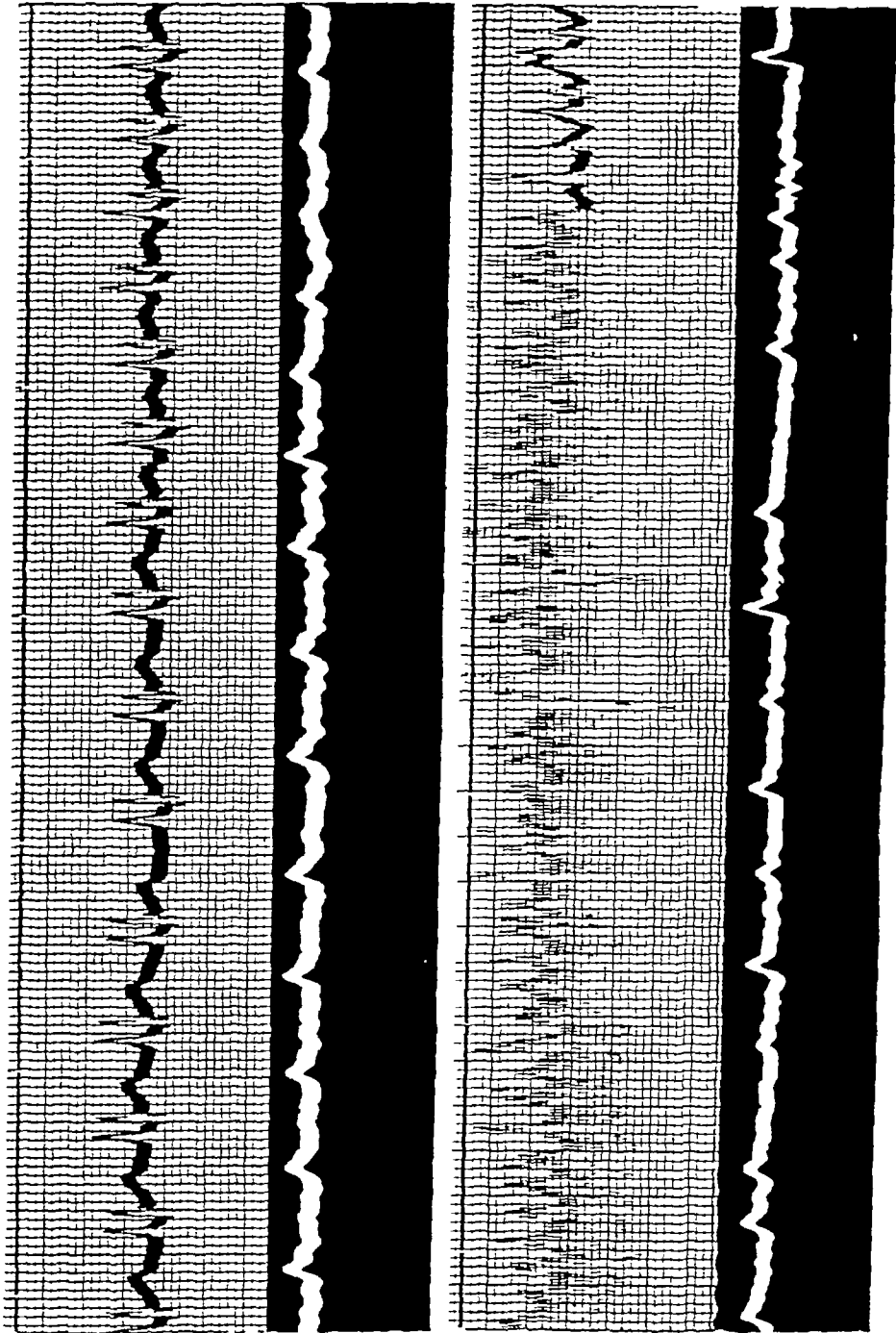
X-ray photographs of the heart taken at a distance of 2 meters were made according to the method described by Stewart for obtaining photographs of the hearts of dogs under uniform conditions. The x-ray shadows of the heart were traced on thin paper and the areas measured with a planimeter (13).

#### *Plan of observations*

The day after the preliminary operation the following observations were made. The dogs, in a basal metabolic state, lay quietly on the table without anesthesia. A set of observations consisting of an x-ray photograph of the heart, samples of arterial and of mixed venous blood, measurement of oxygen consumption, and electrocardiograms together with simultaneous femoral pulse tracings, was taken during the period of normal cardiac rhythm, hereafter called the "period of normal rhythm." Taking a complete set of observations required usually 30 to 45 minutes. The right auricle was then made subject to fibrillation. At the end of approximately one hour, designated the "period of auricular fibrillation," while the auricles were still fibrillating, a second set of observations was made. Then, while this rhythm was maintained by continuing faradic stimulation, tincture of digitalis (Upsher Smith) was injected intravenously in an amount calculated to be 25 to 30 per cent of the lethal dose.<sup>1</sup>

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<sup>1</sup> This amount was chosen because Robinson and Wilson (14) found the therapeutic dose of digitalis for cats to be 30 per cent of the calculated lethal dose, and because Cohn (15) found that the dose for cats must be multiplied by the factor 1.16 to arrive at a comparable quantity for dogs. We have accordingly injected this amount of the tincture. To several dogs we administered digifoline (Ciba) 0.5 cc per kilogram of body weight (16, 17). The same phenomena resulted irrespective of the preparation that was given.



X Y

A B

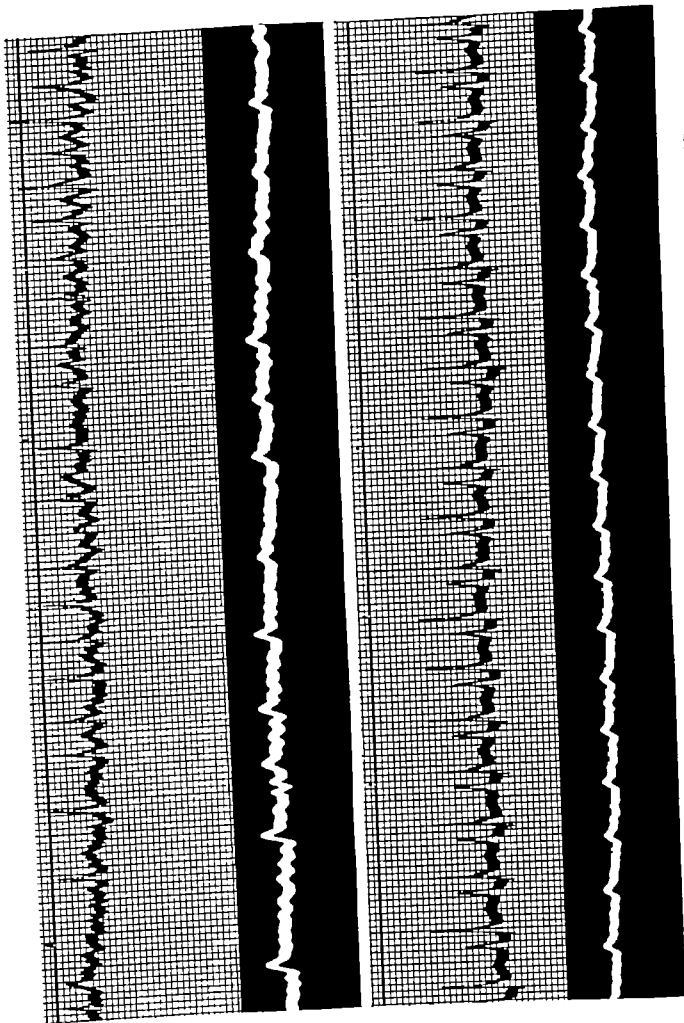


FIG 2 IN THIS FIGURE ARE SHOWN THE RECORDS OF DOG 231 TAKEN ON APRIL 14 1927  
*A* was taken when the rhythm was normal *B* during and after auricular fibrillation had been present 71 minutes *C* 200 minutes after the onset of auricular fibrillation and 105 minutes after the injection of tincture of digitalis (Upsher Smith) 2.0 cc. intravenously and *D* 81 minutes after the return to the normal rhythm and 210 minutes after injecting digitalis *Y* is the electrocardiogram (Lead II) and *Z* the femoral pulse tracing. A short time interval elapses between the beginning of the QRS complex of the electrocardiogram and the corresponding femoral pulse due partly to the time required for transmission of impulses from the heart to the femoral artery and partly to that from the rubber cuff to the Frank capsule. In the femoral pulse tracing taken during auricular fibrillation (*B* and *C*) attention is called to the variation in the excursion of the femoral pulse from beat to beat. In this figure divisions of the ordinates equal 10-4 volts divisions of the abscissae equal 0.04 of a second. The original curves are sharply contrasted black and white; no half tones are lost by the method of reproduction. The curves are reproduced full size.

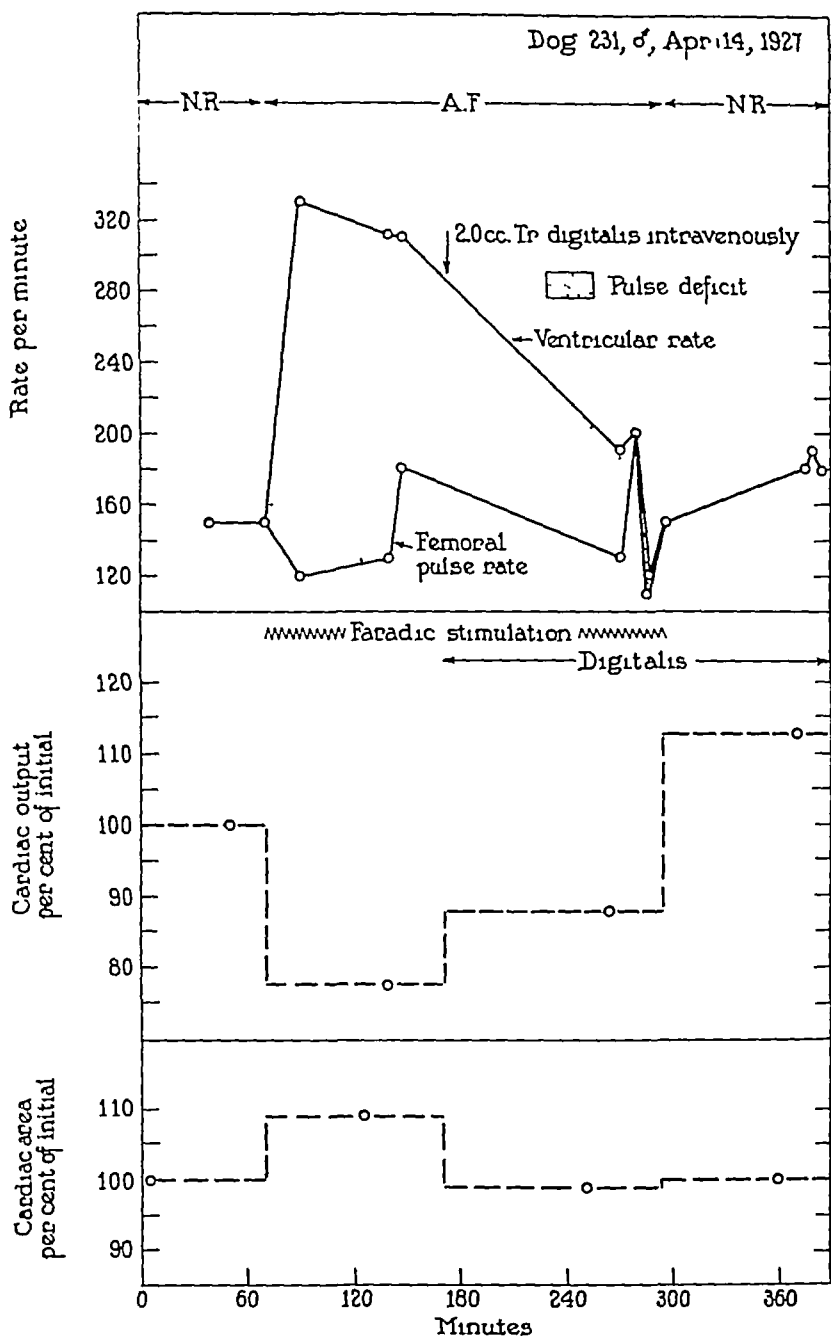


FIG 3 IN THIS FIGURE IS SHOWN THE EFFECT OF GIVING DIGITALIS ON CARDIAC OUTPUT, CARDIAC SIZE, VENTRICULAR RATE AND FEMORAL PULSE RATE PER MINUTE IN DOG 231 (APRIL 14, 1927) DURING ARTIFICIAL AURICULAR FIBRILLATION

In this figure as well as in Figure 4, the points on the curves show the exact time (abscissa) at which each observation was made. In order to bring into relief the relations between the various situations at these times, the curves are drawn in dotted straight lines as though these relations existed throughout the various periods. In this figure and in Figure 4, N R indicates normal rhythm and A F, auricular fibrillation.

TABLE 1  
The effect of going digitalis on the cardiac output, cardiac size and ventricular rate in dogs subject to artificial auricular fibrillation

Dog number and sex	Date	Time with reference to faradic stimulation	Time with reference to administration of digitalis ††	Weight kgs.	O <sub>2</sub> content		Arterio-venous oxygen difference	O <sub>2</sub> consumption cc. per minute	Cardiac output cc. per minute	Cardiac output per cent of initial	Cardiac area sq. cm.	Cardiac area per cent of initial	O <sub>2</sub> cap-acity	Ven-tricular rate*	Corre-sponding femoral pulse rate*	Diet allis	Rhythm of heart
					Arte-rial blood	Mixed venous blood											
231 ♂	1937 Apr 14	0-65 B	B	10.45	25.24	17.88	7.36	82	1112	100	36.5	100	26.05	150	150	cc.	NR †
					25.49	15.77	9.72	84	864	78	39.6	109	26.05	150	150		
		60-75 A	B		24.62	15.30	9.32	91	976	88	36.0	99	24.95	190	130	20 †	AF
					23.77	16.71	7.06	89	1261	113	36.5	100	26.19	180	180		
		64-89 E	190-230 A		15.79	9.63	6.16	95	1542	100	40.2	100	15.72	100	100	NR.	NR.
					16.24	5.47	10.77	119	1105	72	42.5	106	16.53	270	170		
	Apr 21	0-45 B 60-100 A	B	15.57	5.38	10.19	125	1227	80	39.5	98	15.95	180	170	4.66 †	A.F. NR	
				15.46	6.52	8.94	104	1163	75	37.6	94	16.02	170	170			





TABLE 1 (continued)

Dog number and sex	Date	Time with reference to faradic stimulation	Time with reference to administration of digitalis††	Weight kgm.	O <sub>2</sub> content		Arterio-venous oxygen difference	O <sub>2</sub> consumption	Cardiac output	Cardiac output per cent of initial	Cardiac area	Cardiac area per cent of initial	O <sub>2</sub> capacity	Ven-tricular rate*	Corre-sponding femoral pulse rate*	Digit alis	Rhythm of heart	
					Arte-rial blood	Mixed venous blood												
234 ♀	1937 Apr 28	minutes	minutes	15.2	vol% per cent	vol% per cent	vol% per cent	cc. per minute	cc. per minute	per cent	sq. cm.	per cent	per cent	vol% per cent	per minute	per minute	cc.	N R.  A.F.  A.F. N R.
		0-49 B	45-75 A		24.86	14.07	9.99	113	1131	100	50.0	100	100	25.69	200	200		
		60-80 A	153-185 A		25.49	13.73	11.76	104	884	78	53.0	106	106	26.32	190	180		
		140-170 A 60-95 E			24.86 24.03	17.96 16.44	6.88 7.59	108 112	1569 1449	138 127	48.0 46.3	96 93	96 93	24.32 25.98	220 190	220 190	80‡	

|| B = before beginning stimulation, A = after beginning stimulation, E = after ending stimulation see text "Plan of Observations"

†† B = before injecting digitalis, A = after injecting digitalis

\* In this table as well as in Table 2, the ventricular rate and the corresponding femoral pulse rate were counted in the electrocardiographic record and simultaneous femoral pulse tracing

† N.R. = normal rhythm

‡ A.F. = auricular fibrillation

§ Tincture of digitalis (Upsher Smith) given intravenously

§ Digifoline (Ciba) given intravenously

\*\* In this dog observations were made during a second "period of auricular fibrillation with digitalis"

Approximately one hour later, during the "period of auricular fibrillation with digitalis," the auricles still fibrillating, a third set of observations was made. Stimulation of the auricles was then discontinued and the rhythm of the heart became normal again. After another hour, designated the "period of normal rhythm with digitalis," a fourth set of observations was made. Deviation from this schedule, whenever it occurred, is shown in the tables.

There are complete observations in four dogs. The results secured in the case of dog 231 serve to illustrate the course of events (Table 1, Figure 3). When the heart was beating normally at a rate of 150 per minute its output was 1112 cc per minute, and the area of the heart measured 36.5 sq cm (Table 1, April 14, 1927). One hour after the onset of auricular fibrillation and while this rhythm was still present, the ventricular rate being 300 to 330 (Figure 2) and the femoral pulse rate 120 to 180 per minute, the cardiac output fell to 864 cc per minute or 78 per cent of its initial value (Table 1, Figure 3), and the area of the heart measured 39.6 sq cm, an increase to 109 per cent of its initial size. While the auricles were fibrillating, tincture of digitalis (Upsher Smith) 2.0 grams was given intravenously. Eighty-five minutes later, under the influence of digitalis, the ventricular rate decreased further from 200 to 120, and the corresponding femoral pulse rate from 200 to 110 per minute, the pulse deficit being smaller, the heart was, in short, still beating irregularly but more slowly and more forcibly, there being only a few ineffective beats. The cardiac output *increased* to 976 cc per minute (88 per cent of its initial value) and its size *decreased* to 36.0 sq cm (99 per cent of its initial size). Then stimulation of the auricles was discontinued and the rhythm of the heart again became normal. One hour later the cardiac rate was 190 to 200 per minute, the heart still being under the influence of digitalis. Since there was no longer a pulse deficit, the cardiac output increased to 1261 cc per minute (113 per cent of the initial) though the area of the heart remained unchanged (36.5 sq cm, 100 per cent). When the observations were repeated one week later (April 21, 1927) similar results were obtained (Table 1), with this exception that cardiac output and cardiac size both diminished when the rhythm of the heart became regular again in the final (fourth) period.

*Summary* With the onset of auricular fibrillation the cardiac output decreased and the heart dilated. When digitalis was given the size diminished and the output increased, that is to say, the capacity of the heart to expel blood increased. The results in the three other dogs were similar to these. The most frequent effect observed after the end of stimulation, when the rhythm again became regular, was further decrease in the size of the heart and a decrease in output. In other instances, as in the one described in detail, cardiac size remained unchanged from that in the fibrillatory state and the cardiac output increased.

TABLE 2  
*The effect of going digitalis on the relative blood flow, cardiac size and ventricular rate in dogs subject to artificial auricular fibrillation*

Dog num- ber and sex	Date	Time with reference to intracardiac stimulation II	Time with reference to administra- tion of digitalis II	Weight kgm	O <sub>2</sub> content		Arterio- venous oxygen differ- ence *	Blood flow per cent of initial	Cardiac area per cent Initial	O <sub>2</sub> cap- acity	Ven- tricular rate	Corre- sponding femoral pulse rate	Digit- alis †	Rhythm of heart		
					Arte- rial blood	Mixed venous blood										
226 ♀	February 11, 1927	0-13 B	B	14.75	11.25	8.17	mLf 2.99	100	45.2	mLf 11.81	200	200	cc.	N.R. ‡		
										190	190	200				
		70-105 A	B		11.80	6.72	5.08	59	50.7	112	12.04	350		160	28	A.F. §
										340	160	350				
		170-189 A	60-79 A		11.07	7.70	3.37	89	47.0	12.22	270	270		A.F.		
										210	210	210				
		60-80 E	150-170 A		11.11	7.08	4.03	74	42.8	95	12.06	190		190		N.R.
										200	200	190				
214 ♀	December 16 1926	0-50 B	B	13.88	10.47	7.14	3.33	100	44.4	9.84	230	230	30	N.R.		
										63	50.4	113			340	190
		208-268 A	60-120 A		10.52	6.91	3.61	92	44.8	101	10.36	200		200		A.F.
					10.89	6.30	4.59	72	41.6	94	11.15	210		210		

TABLE 2 (continued)

Dog number and sex	Date	Time with reference to famelic stimulation	Time with reference to administration of digitalis ††	Weight	O <sub>2</sub> content		Arterio-venous oxygen difference *	Blood flow per cent of initial	Cardiac area	Cardiac area per cent of initial	O <sub>2</sub> capacity	Ven tricular rate	Corresponding femoral pulse rate	Digitalis ‡	Rhythm of heart
220 ♀	January 19, 1927	minutes	minutes	kgm	Arterial blood	Mixed venous blood	mM	per cent	sq cm	per cent	mM	per minute	per minute	cc	N R
		0-22 B	B	10 2	11 10	8 36	2 74	100	34 3	100	11 21	130	130		
		60-90 A	B		11 21	6 84	4 37	62	37 8	110	11 40	140	140		A F
		180-210 A	60-90 A		10 29	6 53	3 76	73	34 6	100	10 40	160	150	2 3	A F
		210-270 A ‡	135 A		10 74	6 92	3 82	72			11 45	200 ‡	190		A F
223 ♀	February 3, 1927	75-100 E	360-385 A		11 08	6 63	4 45	61	31 3	91	11 39	180	180		N R
												190	190		
		0-40 B	B	11 0	11 09	8 84	2 25	100	39 2	100	11 88	160	160		N R
		40-60 A	B		11 70	6 77	4 93	46	42 5	109	11 90	340	180	2 0	A F
		115-150 A	47-82 A		11 04	6 96	4 08	55	41 9	106	12 39	180	180		A F
		60-80 E	137-157 A		11 47	6 50	3 97	57	40 9	104	12 17	170	170		N R

|| B = before beginning stimulation, A = after beginning stimulation, E = after ending stimulation, see text "Plan of Observations"

†† B = before injecting digitalis, A = after injecting digitalis

\* Oxygen removed from each liter of blood

† Tincture of digitalis (Upsher Smith) given intravenously

‡ N R = normal rhythm

§ A F = auricular fibrillation

‡ In this dog observations were made during a second "period of auricular fibrillation with digitalis"

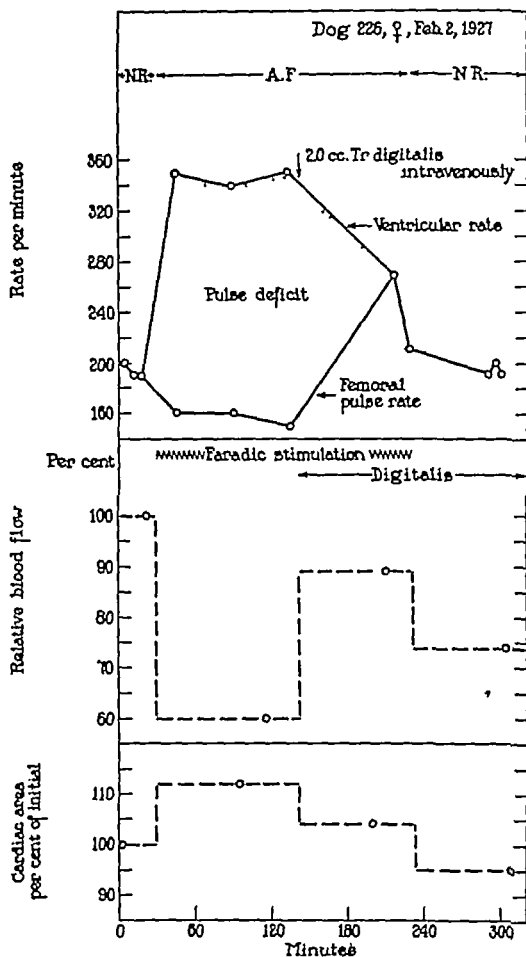


FIG 4 IN THIS FIGURE IS SHOWN THE EFFECT OF GIVING DIGITALIS ON THE RELATIVE BLOOD FLOW, CARDIAC SIZE, VENTRICULAR AND FEMORAL PULSE RATES PER MINUTE IN DOG 226 DURING ARTIFICIAL ATRICULAR FIBRILLATION

In four untrained dogs similar observations were made (Table 2). The arteriovenous oxygen difference was measured representing, as it does, the oxygen consumed by the tissues per liter of blood, and from this the relative blood flow<sup>2</sup> (6) has been calculated, the control period being placed at 100, the results in all these measurements are similar to those in which the cardiac output was measured. Dog 226 (Table 2, Figure 4) may serve to illustrate the course of events. There was decrease in relative blood flow and increase in cardiac size when the cardiac mechanism was that of auricular fibrillation, increase in output and decrease in size when digitalis was given during this rhythm and finally, decrease in output and decrease in cardiac size with the return of the normal rhythm. The results in the three other dogs are similar.

*A note on the effect of giving digitalis to a dog in the presence of edema artificially induced*

In dog 265, edema of the extremities occurred as a result of taking sodium bromide<sup>3</sup> (18, 19, 20, 21). The fluid intake was uniform, being 1500 cc a day. The diet consisted of milk and eggs given by stomach tube at the same time each evening. The output of urine was measured. The dog was trained in the use of the spirometer for measuring oxygen consumption. All measurements were made when the dog was in a basal metabolic state. On November 27, 1928, when edema appeared, the cardiac output measured 1550 cc per minute and the area of the heart 48.3 sq cm, the left and right ventricular excursions measured in moving x-ray films (1) 2.8 mm and 3.4 mm respectively (Table 3, Figure 5). Tincture of digitalis (Upsher Smith) 2.7 cc was given intravenously to this dog after these measurements had been made. The output of urine increased, the dog lost weight, and became free of edema on November 30. Twenty-four hours after the administration of digitalis, cardiac output *increased* to 2100 cc (136 per cent of the initial output), cardiac size decreased to 47.5 sq cm (89 per cent) and the left and right ventricular excursions both increased (4.8 mm and 4.8 mm respectively). Two days later still, when edema had quite disappeared, the cardiac output *increased* still further to 2550 cc (173 per cent), cardiac size dimin-

<sup>2</sup> "Relative blood flow" is a ratio between some state of flow and another, for example, between the arteriovenous oxygen difference per liter of blood in a normal cardiac mechanism and an abnormal one, the metabolism, that is to say, the oxygen consumption, remaining constant.

<sup>3</sup> This dog was given by stomach tube sodium chloride 15.0 grams (10 per cent solution) a day for 5 days. She was then given, in the same manner, sodium bromide 15.0 grams (10 per cent solution) a day for 5 days, when she became drowsy. Four days later (November 27, 1928) edema of the extremities was observed. Water was given in the morning with sodium chloride or sodium bromide, and milk and eggs in the evening to bring the total daily intake of fluid up to 1500 cc. The caloric value of the food intake was constant.

TABLE 3  
*The effect of giving digitalis on the cardiac output, cardiac size and extent of ventricular excursions in dog 265 (9) subjected to edema after taking sodium bromide*

Date	Time with reference to administration of digitalis	Weight kgs.	O <sub>2</sub> content		Arterio-venous oxygen difference	O <sub>2</sub> consumption	Cardiac output	Cardiac output per cent of initial	Car. diast. area * sq. cm.	Cardiac area per cent of initial	Ventricular excursions **		Presence of edema	Dist. of all ***
			Arterial blood	Mixed venous blood							Left	Right		
1928 November 27 November 28 November 30	Before	14.0	18.86	11.47	vol. per cent 7.39	cc. per minute 115	cc. per minute 1550	per cent 100	sq. cm. 48.3	per cent 100	mm. 2.8	mm. 3.4	Yes	cc. 27
	24 hours after	13.4	15.45	10.50	4.95	105	2100	136	47.5	98	4.8	4.8	Yes	
	72 hours after	12.8	15.85	11.57	4.28	110	2550	173	42.4	88	5.2	5.6	No	

\* These x ray photographs were taken at a distance of 34 inches

\*\* Measured from tracings made of the x ray moving films (Cohn and Stewart (1))

\*\*\* Tincture of digitalis (Upsher Smith) given intravenously



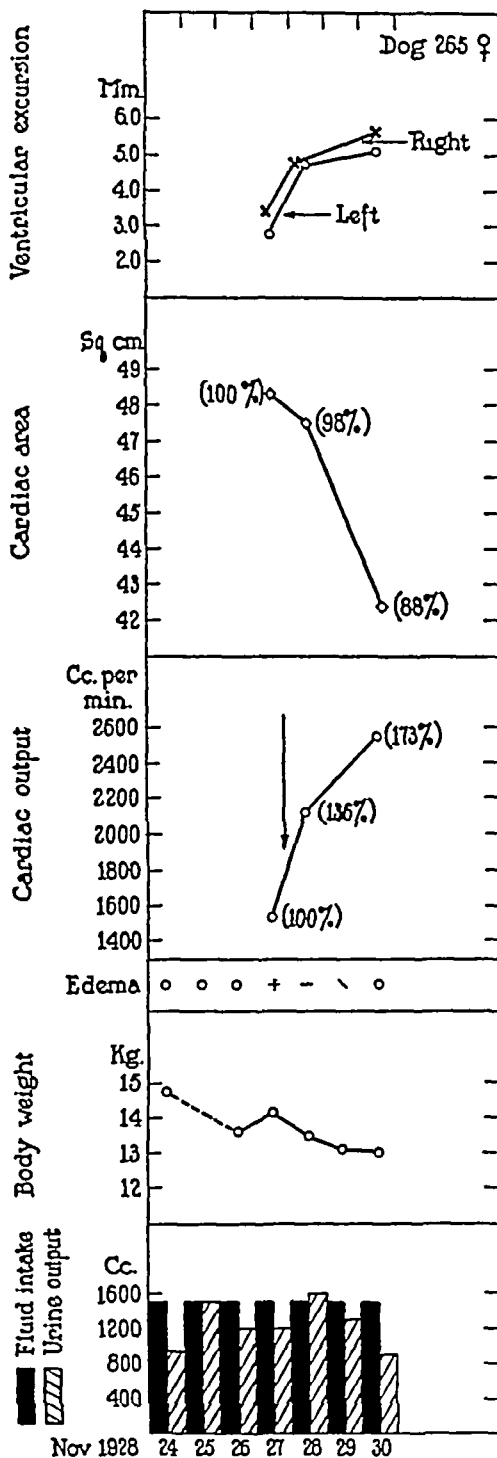


FIG 5 IN THIS FIGURE IS SHOWN THE EFFECT OF GIVING DIGITALIS ON CARDIAC OUTPUT, CARDIAC SIZE AND EXTENT OF VENTRICULAR EXCURSIONS IN DOG 265, SUBJECT TO EDEMA, THE RESULT OF ORAL INGESTION OF SODIUM BROMIDE

In interpreting the symbols relating to the presence of edema, 0 = absent, + = present, - = unchanged, and \ = decreasing

ished further to 42.4 sq cm (88.0 per cent), and the ventricular excursions increased to 5.2 mm (left) and 5.6 mm (right)

*Summary* When digitalis was given to a dog, the subject of edema artificially induced, cardiac output increased, cardiac size diminished and the extent of ventricular excursions increased. The dog lost weight and became free of edema.

#### DISCUSSION

That the effect of giving digitalis to intact normal dogs is to decrease cardiac output and cardiac size and to increase ventricular excursions appears to be a fact (1, 17). Its effect in dogs in which the hearts were enlarged, but in which there was no evidence of heart failure has also been studied. In this situation, as in the normal one, the effect was to decrease cardiac output and cardiac size and to increase the extent of ventricular excursions (2). The effect of giving digitalis has been studied therefore under the following circumstances. First, in normal dogs the hearts of which were normal in size, second, in dogs the subject of muscular hypertrophy following the creation of artificial valvular lesions. In both these, the result is decrease in output. And now there is a case in which information was still lacking, namely, in intact, unanesthetized dogs in which the cardiac output is diminished and the heart dilated due to the action of auricular fibrillation artificially induced. The result is *increase* in cardiac output and *decrease* in cardiac size. Insufficient hearts become smaller, it seems, and, unlike normal hearts in which it decreases, increase their output as the result of a change dependent on the heart itself, and obviously independent of changes in a peripheral mechanism, such as that of the throttle action of the hepatic veins. The outstanding point is this, that at a time when the basic mechanism of the heart beat remained unchanged, the action of digitalis resulted in increase in output, though the rate decreased, occasionally it increased the output still more when the normal rhythm was restored, though the normal did not attain the fibrillatory rate.

In these observations and in the ones published in the third paper (22) parallel phenomena have been studied in dogs and in human beings, namely, the effect of giving digitalis to normal animals and to normal human beings on the one hand, on the other, the effect in dogs in which the cardiac output is diminished and dilatation is present, and in patients the subjects of heart failure, whose hearts are enlarged and their output diminished. The output, both in normal dogs and in normal men, as well as the size, decrease, in diseased human beings and in abnormal dogs during heart failure, increase in output and decrease in size take place. Discussion of these results will be found in a third report (22). It is sufficient to state now that digitalis seems to exert the same essential effects both

in normal and diseased hearts, it decreases cardiac size, its effect on tone, and it increases the extent of ventricular contraction, its effect on contraction (1, 2)

Edema which results in dogs from the oral administration of large doses of sodium bromide is probably not analogous to edema occurring in heart failure. The mechanism of its occurrence is not clearly known. It is presumably the result of shifting of ions in the blood, so that bromide ions are substituted for chloride ions, in consequence of this process, water is retained in the tissues. The course of events following the administration of digitalis in this condition resembles that in heart failure in human beings, namely, the cardiac output increases, the cardiac size decreases and the extent of ventricular excursion increases.

#### SUMMARY AND CONCLUSIONS

1 During auricular fibrillation when the ventricular rate is rapid the cardiac output per minute is less than it is during the normal, slower sinus rhythm (3). In consequence of this abnormal rhythm in intact unanesthetized trained dogs the heart increases in size. This conclusion is based on a larger number of observations than was possible in an earlier paper (4).

2 When the cardiac output is diminished and the heart is dilated due to artificial auricular fibrillation, the administration of digitalis results in increase in cardiac output and decrease in cardiac size.

3 When the normal rhythm returns, the heart being, of course, still under the influence of digitalis, the output either increases, the size remaining unchanged (from that in the fibrillatory state), or both output and size decrease.

4 The observations show, as do the ones next to be reported, that digitalis has the same action in normal and in pathological hearts, it decreases cardiac size (an effect on tone). The amount of the cardiac output which results from this action depends upon the initial size of the heart, it decreases in normal hearts, and increases in dilated ones.

5 In a dog, the subject of edema due to taking sodium bromide, the administration of digitalis increased cardiac output, decreased cardiac size and increased the extent of ventricular excursions.

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# STUDIES ON THE EFFECT OF THE ACTION OF DIGITALIS ON THE OUTPUT OF BLOOD FROM THE HEART III<sup>1</sup>

## PART 1 THE EFFECT ON THE OUTPUT IN NORMAL HUMAN HEARTS

## PART 2 THE EFFECT ON THE OUTPUT OF HEARTS IN HEART FAILURE WITH CONGESTION, IN HUMAN BEINGS

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### PART I

#### THE EFFECT ON THE OUTPUT IN NORMAL HUMAN HEARTS

The action of digitalis when given to human beings is still incompletely understood. Investigations, in recent years (1, 2, 3, 4), which have been directed toward elucidating its influence on the behavior of the heart have led to a certain degree of confusion. The analysis of the results which have been obtained shows that inferences which were drawn were in part inexact, due to utilizing the results obtained in one situation, in the normal hearts of dogs for example or in those of human beings, in an explanation of the condition of disease. But beside difficulties in interpretation of this sort, error has also resulted from the use of insufficiently tested methods. This defect in the case of human beings applies, it seems, to the use of the method of Field, Bock, Gildea and Lathrop (5) by certain investigators (1, 6), and to that of Henderson and Haggard (7) by others (8). The opportunity is still open, therefore, to study the minute volume output<sup>2</sup> of human hearts under the influence of digitalis when they are normal and also when they are the subjects of heart disease. This study is the more important since the result, observed in analyzing the effect on normal hearts, differs, as it turns out, so profoundly from that which supposedly obtains in enlarged diseased ones. If the two are really the same, fundamental conceptions concerning the circulation in heart failure require radical revision.

In this study digitalis was given to six normal individuals on seven occasions. Observations were made of its effect on cardiac output, on

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<sup>1</sup> A preliminary report of these observations appeared in the *Proc. Soc. Exp. Biol. and Med.*, 1931, xxix, 207 and 209.

<sup>2</sup> An abstract of these studies was read before the American Society for Clinical Investigation, May 2, 1932.

<sup>3</sup> It is always the volume output of blood per minute to which reference is made when the word "output" is used.

cardiac size, on arterial blood pressure, on venous blood pressure, on vital capacity, on cardiac rate, and on the electrocardiogram

Measurements of cardiac output were made by the acetylene method of Grollman (9) which, so far as can now be judged, gives reasonably reliable results, for the cardiac output in man measured by it agrees with the amount estimated simultaneously by a direct method (10, 11). Our results, at all events, agree with those reported by Grollman (12)<sup>4</sup>. Estimations of oxygen consumption for use in calculating the cardiac output were made with a Benedict-Roth spirometer. The diastolic size of the heart was traced from x-ray photographs taken at a distance of 2 meters, the subject being placed in a standing position, on thin paper and its area measured with a planimeter as suggested by Levy (13). Estimations of blood pressure were made by the auscultatory method. The three standard leads of the electrocardiogram were taken. Measurements of venous pressure were made by direct methods which will be described later. The vital capacity was measured in a spirometer.

#### PLAN OF OBSERVATIONS

After eating a moderate supper the subjects went to bed in the hospital the night before the observations began, and remained there at rest until 24 hours after the administration of digitalis. To assure their being in a basal metabolic state they were given small carbohydrate meals. The volume output was measured at 2, 4, 9, 12 and 15 hours after giving digitalis. The meals were taken just after the 4 and 15 hour measurements. Since from Grollman's observations three hours are required to return to a basal minute volume output (14), though the basal metabolic rate may still be elevated 1 or 2 per cent (15), this distribution made it certain that the measurements were proper. No water was given for three hours before each observation in order to avoid the effect of ingestion of fluid (16). The Gatch frame of the bed was raised one half-hour before observations so that subjects reclined at an angle of 135 degrees because Grollman found that mixing the gases during rebreathing takes place more satisfactorily in this position. Patients suffering from heart failure, moreover, often find it difficult or impossible to lie flat. We wished the two sets of investigations to be comparable from this point of view.

Counts of the pulse rate were made at intervals of a few minutes. At the end of a half-hour, subjects rebreathed from a bag containing a mixture of acetylene and air. Two proper samples of gas were taken for analysis and for calculation of the arteriovenous oxygen difference. The oxygen consumption was then estimated. Electrocardiograms were

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<sup>4</sup> We wish to express our thanks to Dr. E. K. Marshall for his kindness in placing at the disposal of one of us (H. J. S.) the facilities of his laboratory to learn this method, and to Dr. Arthur Grollman for his kindness in teaching him his method and discussing our data with him.

taken, followed by measurement of arterial and venous pressures and of vital capacity. Subjects were then wheeled in a chair to the roentgen laboratory to secure x ray photographs of the heart. After being weighed they returned to bed to await taking the next observations. Records were made of the body temperature (rectal). The temperature of the room in which the studies were made was kept approximately constant (17). Analysis of the samples of gas for concentrations of carbon dioxide, oxygen and acetylene was made in a modified Haldane apparatus (9).

Observations were made immediately before the administration of digitalis, at the intervals stated during the first 24 and at 24 hour intervals until the return of the initial cardiac output. The subjects had been made familiar with the procedures and were not disturbed by them. After the first 24 hours they attended to their usual daily routines except on days of observation when they remained in bed to assure their being in a basal metabolic state. The initial cardiac output in all except one was normal (Subject 4) and agreed with previous measurements in each case.

### *Observations*

Digitalis in the form of digitan (Merck) 0.8 to 1.0 gram was given by mouth in a single dose. No one experienced nausea or vomiting.

*Effect on cardiac minute volume output.* The initial cardiac output in Subject 1 measured 3.84 liters per minute or 2.17 liters per minute per square meter of body surface (Table 1, Figure 1). Two hours after taking digitalis 0.9 gram the output remained unchanged, 4 hours afterward it fell to 3.68 liters per minute. Progressive decrease followed. The lowest point was recorded 24 hours later when it measured 2.54 liters per minute or 1.44 liter per minute per square meter of body surface, equal to 66 per cent only of its initial value. Twenty-four hours later still, the output was 3.01 liters per minute, that is to say, 81 per cent of the initial amount. From this time it returned slowly toward normal, one week later it was 3.40 liters per minute (88 per cent of normal) and after another it was still low.

The results in the 5 other persons were similar (Tables 1 and 2, Figures 2, 3 and 4), with this exception, that the effect of the drug was of shorter duration. The earliest effects were found after 4 to 12 hours, the maximum ones in 4 to 24 hours. At the time of the maximum effect the output was reduced to 60 to 85 per cent of the initial amount.

The normal resting arteriovenous oxygen difference in Subject 1 was 58.4 cc. per 100 cc. blood. Two hours after the administration of digitalis 0.9 gram it remained unchanged, four hours afterward a slight increase occurred (Table 1). Progressive increase took place so that 24 hours later it was 88.0 cc. per 100 cc. blood. The arteriovenous oxygen difference then returned slowly toward its initial value, although at the end of two weeks this had not been attained.



TABLE I  
Effect of giving digitalis on cardiac output and cardiac size in normal individuals

Patient number	Age	Date	Weight kgm	Height cm	Body surface sq m	Oxygen consumption cc per minute	Arterio-venous oxygen difference cc.	Cardiac output liters per minute	Cardiac output percent of initial	Cardiac area sq cm	Cardiac area percent of initial	Cardiac rate minutes	Cardiac output cc per beat	Arterial pressure mm Hg	Summary of effect on electrocardiogram	Pharmacological action	Time with respect to onset of action
1 ♂	34	1931 March 4	69.9	165.5	1.77	224	59.4	3.81	100	105.3	100	80	48	112/83	T waves became of lower voltage but remained positive. P waves changed form. The ventricular rate became slower.	0.9	Before 2 hours after 4 hours after 9 hours after 12 hours after 15 hours after 18 hours after 18 hours after 3 days after 4 days after 5 days after 7 days after 16 days after
2 ♂	31	February 10	73.9	175.5	1.89	230	62.2	3.81	100	110.5	100	58	66	95/60	T <sub>2</sub> became negative and was still diphasic on March 26 1931. The ventricular rate became slower.	0.8	Before 4 hours after 9 hours after 12 hours after 15 hours after 18 hours after 3 days after
3 ♂	20	February 21	82.0	183.0	2.04	230	59.9	3.93	100	119.8	100	54	73	100/65	T <sub>2</sub> became of lower voltage but remained positive. The ventricular rate became slower.	0.8	Before 2 hours after 4 hours after 9 hours after 15 hours after 23 hours after 19 hours after 3 days after
4 ♂	31	June 10	81.0	165.5	1.69	169	63.8	2.65	100	136.3	100	70	38	120/90	T <sub>2</sub> which were positive at beginning became — T <sub>2</sub> and diphasic. T <sub>2</sub> became slower. T <sub>2</sub> became negative and then diphasic.	1.0	Before 12 hours after 24 hours after 34 hours after 48 hours after

\* Patient was not in true posterior-anterior position

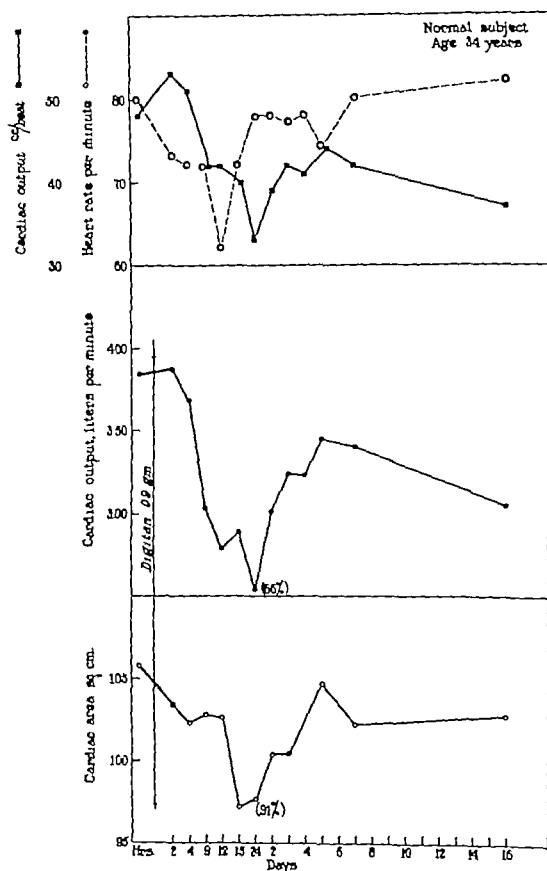


FIG 1 IN THIS FIGURE IS REPRESENTED THE EFFECT OF GIVING DIGITALIS ON CARDIAC OUTPUT CARDIAC SIZE AND CARDIAC RATE PER MINUTE IN SUBJECT NUMBER 1

# ACTION OF DIGITALIS III

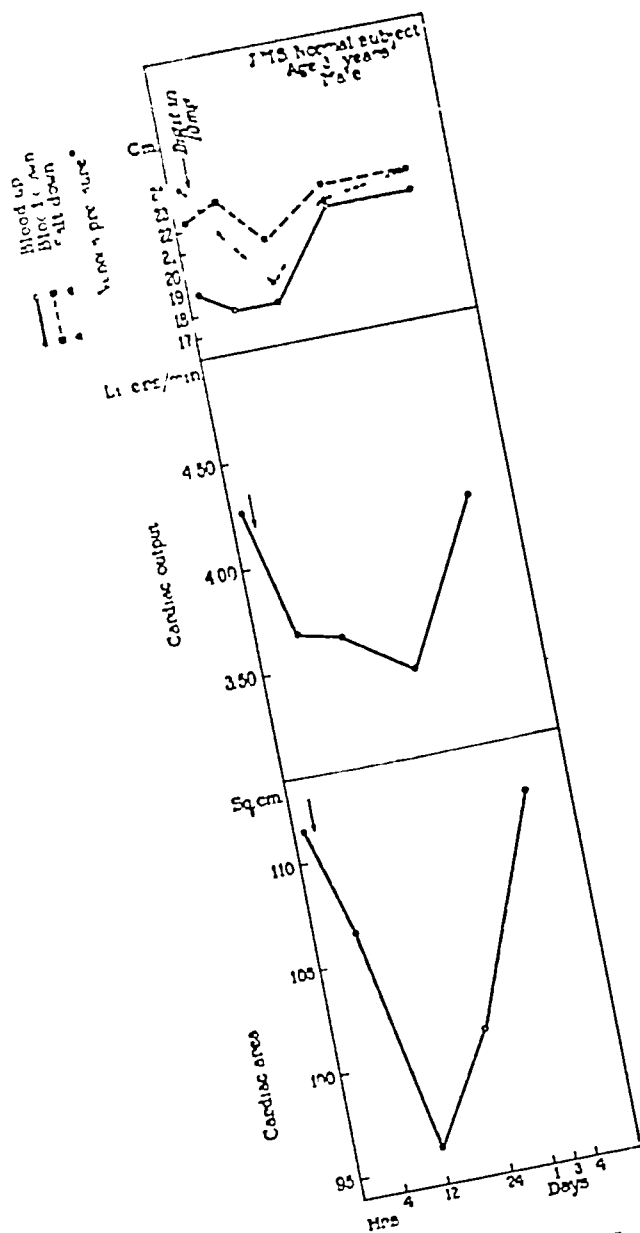


FIG. 2 IN THIS FIGURE IS REPRESENTED THE EFFECT OF DIGITALIS ON CARDIAC OUTPUT, CARDIAC SIZE AND VENOUS PRESSURE NUMBER 2

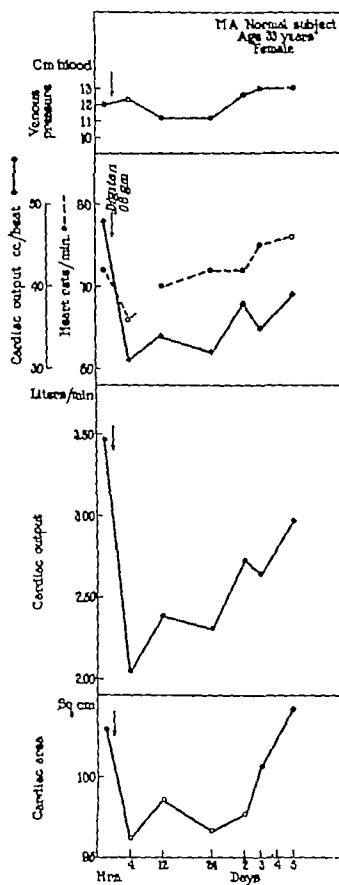


FIG 3 IN THIS FIGURE IS REPRESENTED THE EFFECT OF GIVING DIGITALIS ON CARDIAC OUTPUT CARDIAC SIZE CARDIAC RATE AND VENOUS PRESSURE IN SUBJECT NUMBER 6

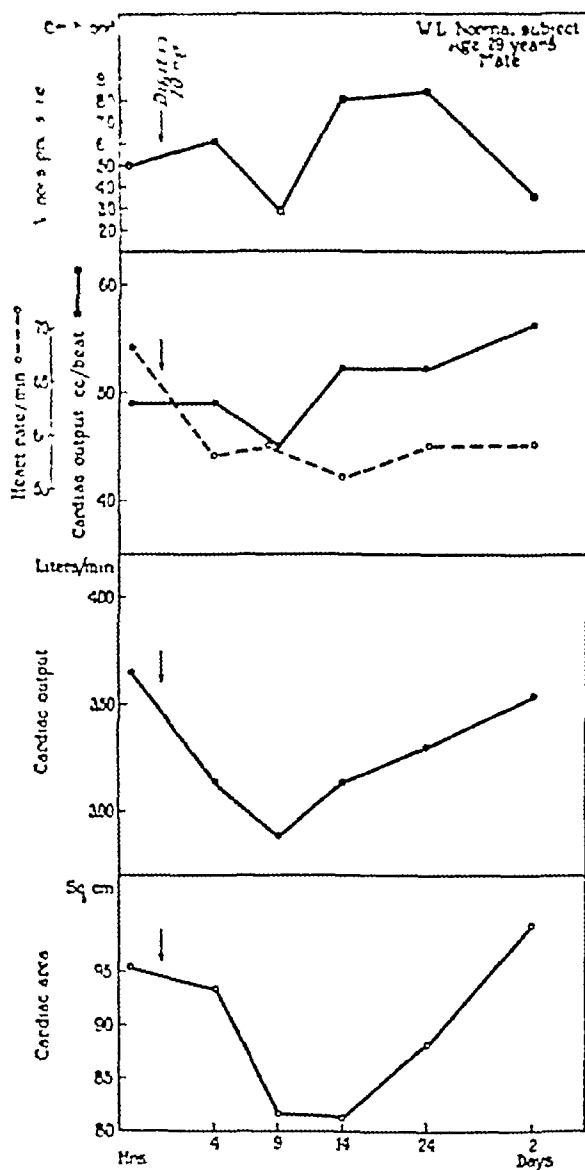


FIG. 4. IN THIS FIGURE IS REPRESENTED THE EFFECT OF GIVING DIGITALIS ON CARDIAC OUTPUT, CARDIAC SIZE AND VENOUS PRESSURE IN SUBJECT NUMBER 5.

TABLE 2

### Effect of evening digitalis on cardiac output, venous pressure and vital capacity in normal individuals

Sub- ject	Age	Date	Weight	Height	Body sur- face	Oxy- gen con- sump- tion	Arterio- venous oxygen differ- ence	Cardiac output	Cardiac output per cent of initial	Cardiac output ml. per sq. m. per minute	Cardiac area	Cardiac sec-les per cent of initial	Heart rate	Cardiac output cc. per beat	Arterial pressure mm. Hg	Yemo- c pressure	Vital capac- ity	Body temper- ature (rectal)	Digit- an	Summary of effect on electro- cardi- ogram	Time with re- ference to digitalis admin- istration	
5	28	1938 January 20	68.7	170.7	cm. sq. m.	cc. per min- ute	cc.	liters per min.	per cent	liters per min.	sq. m.	per cent	min- utes	cc. per beat	mm. Hg	cm. blood	cc.	liters	P	normal	Before	
5	28	January 21	68.5		1.70	231	73.5	3.14	86	1.75	103.8	93.6	68	64	108/70	8.0	4300	98.5	1.0	T12 became of lower voltage	4 hours after 9 hours after 14 hours after 21 hours after 48 hours after	
5	28	January 22	68.5		1.70	231	73.0	3.14	86	1.75	103.8	93.6	68	64	108/70	8.0	4300	98.5				
5	28	January 23	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	January 24	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	January 25	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	January 26	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	January 27	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	January 28	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	January 29	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	January 30	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	January 31	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	February 1	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	February 2	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	February 3	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	February 4	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	February 5	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	February 6	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	February 7	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	February 8	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	February 9	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	February 10	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	February 11	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	February 12	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	February 13	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	February 14	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	February 15	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	February 16	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	February 17	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	February 18	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	February 19	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	February 20	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	February 21	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	February 22	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	February 23	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	February 24	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	February 25	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	February 26	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	February 27	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	February 28	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	February 29	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	March 1	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	March 2	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	March 3	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	March 4	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	March 5	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	March 6	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	March 7	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	March 8	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	March 9	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	March 10	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	March 11	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	March 12	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	March 13	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	March 14	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	March 15	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	March 16	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	March 17	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	March 18	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	March 19	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	March 20	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	March 21	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	March 22	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	March 23	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	March 24	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	March 25	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	March 26	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	March 27	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	March 28	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	March 29	68.5		1.70	230	69.0	3.54	97	1.98	99.4	108	59	60	105/60	8.3	4350	98.8				
5	28	March 30	68.5		1.70	230	69.0	3.54	97													

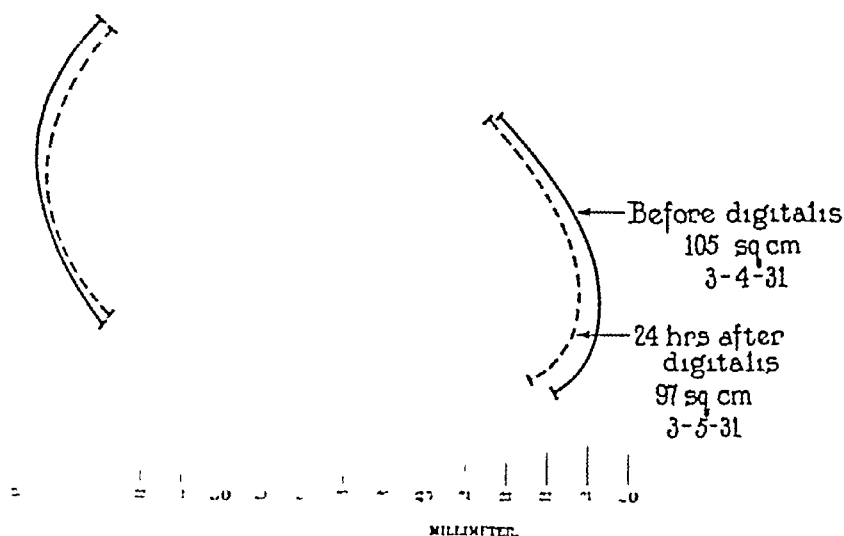


FIG. 5. IN THIS FIGURE IS REPRESENTED THE CHANGE IN SIZE OF THE HEART FOLLOWING THE ADMINISTRATION OF DIGITAN 0.9 GRAM IN SUBJECT NUMBER 1.

The outlines of the heart were traced from the x-ray photographs taken on March 4 and 5 on thin paper and superimposed in the manner shown.

The results in the 5 other cases corresponded to this one (Tables 1 and 2, Figures 2, 3 and 4). The decrease in size varied between 7 and 15 per cent (to 93 and 85 per cent of the initial measurement) and was recorded 4 to 24 hours after the drug was given. As the effect of the drug wore off the heart regained its original size.

*Effect on cardiac rate.* In all six subjects the cardiac rate fell 4 to 14 beats per minute (counted at the time the cardiac output was measured, Tables 1 and 2, Figures 1, 3 and 4). In each instance it returned toward the initial count as the effect of digitalis disappeared.

*Effect on the electrocardiograms.* In all instances change in form of the T waves of the electrocardiograms occurred (Tables 1 and 2). In one

instance (Subject 4)  $T_2$  and  $T_3$  which had been positive became diphasic and  $T_1$ , positive at the beginning, became negative (Table 1) In an other instance (Subject 2)  $T_3$  which was positive became negative as the effect decreased it became diphasic and was diphasic still 38 days later when the last record was made (Table 1 Figure 7) It was upright one year later, when the observations were repeated  $T_3$  was similarly affected

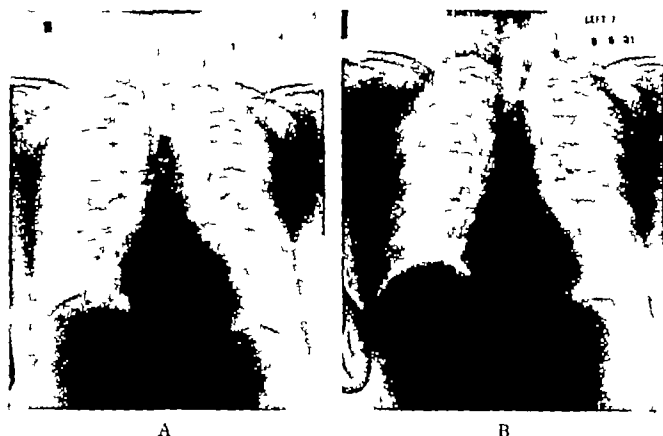


FIG 6 IN THIS FIGURE IS REPRESENTED THE CHANGE IN SIZE OF THE HEART FOLLOWING THE ADMINISTRATION OF DICITAN 0.9 GRAM IN SUBJECT NUMBER 1

Photograph *A* was taken on March 4 1931 immediately before and *B* on March 5 twenty four hours after the administration of the drug

(Table 2, Figure 7) In the four other subjects (Subjects 1, 3, 5 and 6) positive  $T_{123}$  became either smaller or isoelectric (Tables 1 and 2) Alterations of the T waves occurred as early as two and a half hours after administration of the drug (Figure 7)

Increase in auriculoventricular conduction time did not occur nor did abnormal rhythms or premature contractions develop In one instance (Subject 4)  $P_2$  became negative, to resume its earlier form as the effect of digitalis declined and in another instance (Subject 1) the form of the P waves was altered

*Effect on oxygen consumption* The oxygen consumption of each individual remained constant (Tables 1 and 2) The changes in cardiac output which were observed are therefore, not attributable to alterations of oxygen consumption, but to changes in arteriovenous oxygen difference The normal oxygen consumption of one subject (Subject 4) was diminished (Table 1) and remained approximately unchanged in estimations



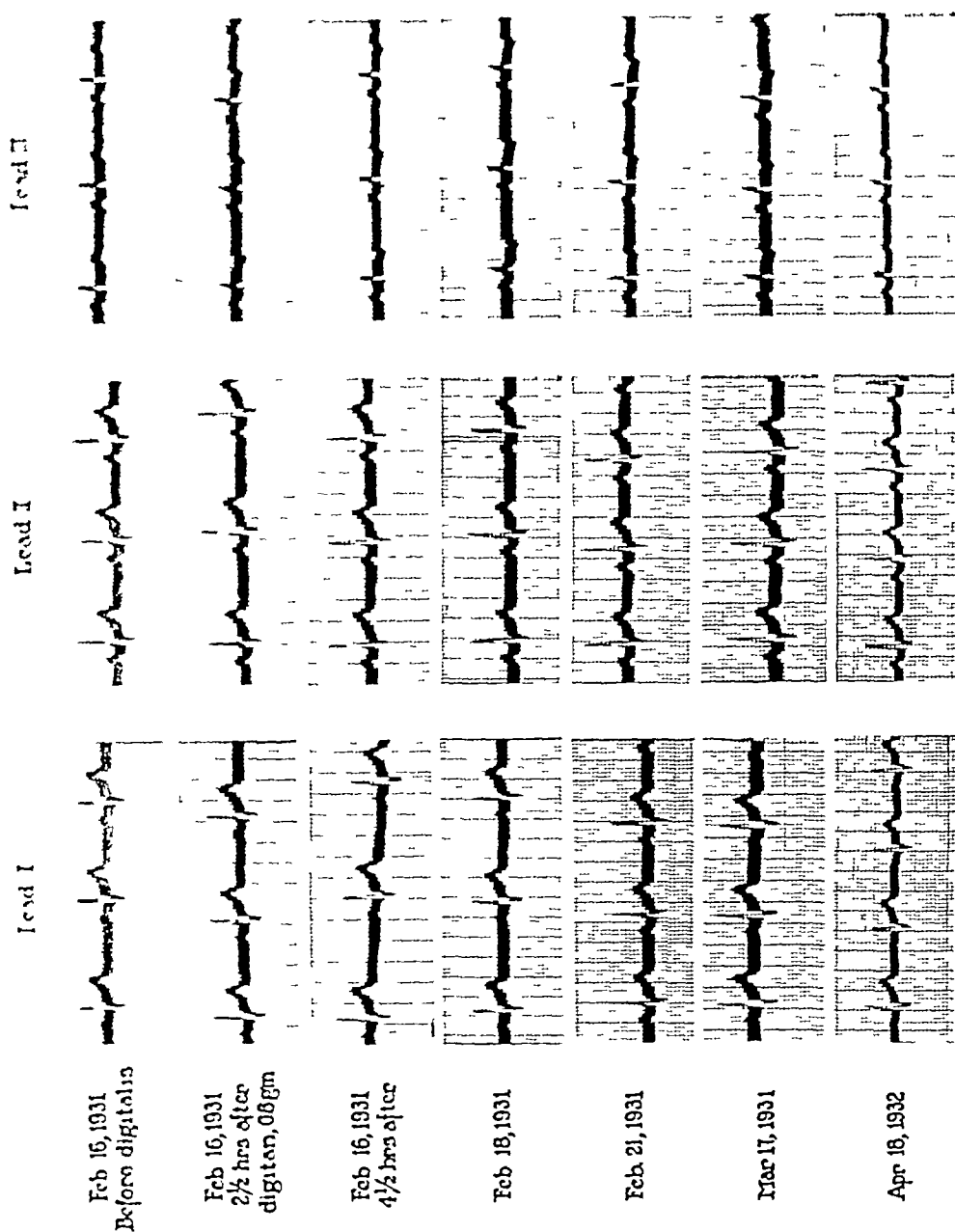


FIG. 7 THE EFFECT OF GIVING DIGITAN 0.8 GRAM ON THE ELECTROCARDIOGRAM OF SUBJECT NUMBER 1 IS SHOWN IN THIS FIGURE

Alterations of the T-waves in Lead III were present 2½ hours after digitalis was given and were still present at the end of 38 days (March 17), when the observations were discontinued. It had, however, regained its usual contour one year later (April 18, 1932). Divisions of the ordinates equal 10-1 volts. Divisions of the abscissae equal 0.04 of a second. The original curves are sharply contrasted black and white, no half tones are lost by this method of reproduction. The electrocardiograms are reduced to one half their natural size.

which were made later. There appeared to be no adequate explanation for his low basal metabolic rate. Since the arteriovenous oxygen difference was normal, the initial cardiac output was less than it should have been for his height and weight (Grollman (12)). But after taking digitalis as in the other subjects, decrease in cardiac output and in cardiac size took place.

*Effect on vital capacity* Significant alterations did not occur in the vital capacities of the three individuals in whom it was measured (Table 2).

*Effect on arterial and venous pressures* Significant alterations either in the systolic or diastolic level of the arterial blood pressure did not occur (Tables 1 and 2).

Estimations of venous pressure whether by direct or indirect methods are unsatisfactory especially from the point of view of comparing the results over long periods of time. Errors arise due to the method but also due to difficulty in selecting a level to represent that of the opening of the venae cavae into the right auricle, and in finding this again in making subsequent estimations. For these reasons the venous pressure was measured in two subjects (Subjects 5 and 6) by the method of Taylor, Thomas and Schleiter (18), but in a third (Subject 2) three methods were compared. In the former method the dry wall of the glass tube apparently hinders the rise of blood. An attempt to learn the pressure in the right auricle by taking the pressure elsewhere in the body is, of course beset with difficulty. The changes which occur in any given region, independent of those elsewhere, make it impossible to be certain that a usual relation continuously exists between the pressures in two veins. It is reasonable to suppose, however, that the direction of change will not be uniformly different. In the absence of a direct method which gives the pressure in the right auricle, the one that has been used yields perhaps the best approximation.

The third subject lay flat on a wide board without pillows at least 15 minutes, his arms at his sides. His venous pressure was measured with the following apparatus. The two arms of a Y tube supplied near their origins with stopcocks were bent at right angles to form manometers, the third one was connected to a needle (Figure 8). The apparatus was sterilized. One of the tubes having a funnel at its upper end was filled with warm sterile normal saline solution, its stopcock being closed. The needle was inserted into a vein. Blood flowed into the open tube. The height of the column was measured from the level of the board ("blood up"). By manipulation or by using a tourniquet, more blood was forced into the manometer. On flowing back into the vein the column came to rest ("blood down"). This stopcock was then closed and the one connected to the tube containing salt solution opened. When this column came to rest, its height also was measured ("salt down"). The needle was always inserted at the same point in the same vein.

Venous pressure estimated in these three ways showed wide variations



(Table 2, Figure 2) The height taken by the "blood up" method was usually lower than by the "blood down," and "salt down" greater than the "blood up," but sometimes it was greater and sometimes less even than the "blood down" measurement. All measurements were made from the level of the board on which the subject lay. To find the correct pressure, a correction must be made to allow for the height of the caval openings above the board. To learn this, x ray photographs are taken at 2 meters, the subject lying flat on his back (Figure 9). It appears that the height sought is approximately 11.0 to 12.0 cm. This, or a similar appropriate figure (found in each case), must be subtracted from the manometric value. If the initial "blood up" measurement is selected, the venous pressure is 19.0 cm, if the "blood down," 22.3 cm, if the "salt down" 23.5 cm (Subject 2).

Detailed attention was given to the matter of the level of venous pressure because it has attained importance from the position taken by Tainter and Dock (4) who believe that decrease in cardiac output in normal dogs after giving digitalis is due to constriction of the hepatic veins. The venous pressure, they found, was low. In the three human cases studied when alterations in cardiac minute output are correlated with the

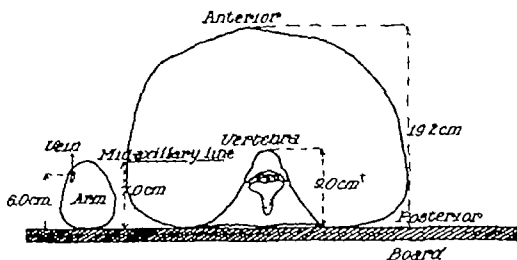


FIG 9 IN THIS FIGURE IS SHOWN THE POSITION OF THE ARM DURING MEASUREMENTS OF VENOUS PRESSURE IN SUBJECT NUMBER 2 (TABLE 2)

The anatomical distances are given

† This measurement was made from an X ray photograph of the chest (lateral view) taken at a distance of 2 meters

height of the venous pressure, it appears from estimations made that no consistent change was observed

In one instance (Subject 5) the minute output diminished to 86 per cent of its normal amount four hours after giving digitalis (Table 2, Figure 4), the venous pressure having increased 1.0 cm (from 5.0 to 6.0 cm). At the end of nine hours when the output fell to 79 per cent the venous pressure measured 2.8 cm, although fourteen hours afterward when the output was again 86 per cent the pressure was high (8.3 cm). Forty-eight hours afterward, when the minute output was normal (97 per cent), the venous pressure was low (3.5 cm). In a second patient



The output of blood from the heart decreased (2) The size of the heart diminished (3) Slight decrease in cardiac rate occurred (4) These effects were at a maximum four to twenty four hours after giving the drug (5) Significant changes did not occur in the levels of arterial and venous pressure (6) Changes in form, sometimes slight, of the T waves of the electrocardiogram occurred in each instance and were present as early as 2½ hours after the drug was given (7) As the effect of digitalis wore off (48 hours to 3 weeks) output, size rate, and the T waves of the electrocardiogram returned toward their initial values (8) A correlation was not established from these data between decrease in cardiac output and change in the level of venous pressure

## PART II

### THE EFFECT ON THE OUTPUT OF HEARTS IN HEART FAILURE WITH CONGESTION, IN HUMAN BEINGS

It has been demonstrated as a fact that giving digitalis decreases the cardiac output and cardiac size in normal human beings. What the effect of its administration is when hearts are in a state of failure is still unknown. Investigations to discover this have, therefore, been undertaken and are now reported.

Seven patients exhibiting signs and symptoms of heart failure were studied. Although Grollman, Proger and Dennig (11) have shown that the acetylene method is adequate for the analysis of cardiac patients exhibiting pulmonary stasis, since the gases in a rebreathing bag come into equilibrium with the blood leaving the lungs within a prescribed time, only such cases were selected as were to all intents and purposes free of pulmonary congestion. The lungs were usually clear at the time the measurements were made. Rarely at the extreme bases behind, a very few râles were heard on deep breathing.

## METHODS

All the patients when studied were at rest in bed. They were taking ward diet, free of salt except that used in cooking. The intake of fluid was except in the case of one patient, limited to 1200 cc a day. In the excepted case it was not limited. Measurements of cardiac output were made as in normal persons by the acetylene method (9, 10, 11). Owing to the difficulty cardiac patients experience in breathing when recumbent, all observations were made one half hour after assuming the sitting position (20) at an angle of 135 degrees, the legs being fully extended. They were made familiar with the procedures beforehand. The vital capacity of the patients was estimated and the volume of gas in the bag adjusted to that amount which each, within a given time, could mix completely (9, 10, 11). This adjustment is necessary because it is impossible, during dyspnea when the vital capacity is diminished, for patients to manage an amount of gas (2400 cc.) as great as can normal individuals.



hypertension the *anatomical* cardiac hypertrophy mitral insufficiency, left ventricular preponderance the *physiological* normal sinus rhythm heart failure of the congestive type (first attack), pulsus alternans

His cardiac output during the period of heart failure measured 2.90 liters per minute<sup>6</sup> on March 17, and 2.88 liters three days later (March 20) (Table 3, Figure 10A). The area of the heart equalled 198.3 sq cm (Table 3 Figures 11 and 12). When digitalis (digitan 1.0 gram) was given (March 20) diuresis occurred (Figure 10A), he lost weight became free of dyspnea and palpitation and the liver became smaller (March 25). On March 21 15 hours after taking the drug the cardiac output increased to 3.54 liters per minute and the size of the heart decreased to 180.2 sq cm (March 22) slowing of the cardiac rate and alterations of the form of the T waves of the electrocardiograms occurred (Table 3, Figures 10A, 11 and 12). As the effect of digitalis wore off the signs of heart failure reappeared (dyspnea palpitation, enlargement of the liver). Two weeks later (April 6) the cardiac output fell to 2.62 liters per minute and the size of the heart increased to 193.6 sq cm. When digitalis was given on three other occasions April 6, May 11 and June 12, results similar to those just described were observed increase in diuresis and in cardiac output, and decrease in cardiac size. As the effect of digitalis declined the signs of heart failure reappeared output decreased and cardiac size increased (Table 3 Figures 10A and 10B).

**Summary** In this patient exhibiting normal cardiac mechanism in the presence of signs and symptoms of heart failure of the congestive type, the volume output of blood from the heart was diminished and the heart was large. On administering digitalis on each of four occasions, output increased and the size of the heart diminished. Changes in the reverse fashion occurred as the effect of digitalis disappeared. Giving digitalis induced diuresis on each occasion and was followed by amelioration of his signs and symptoms.

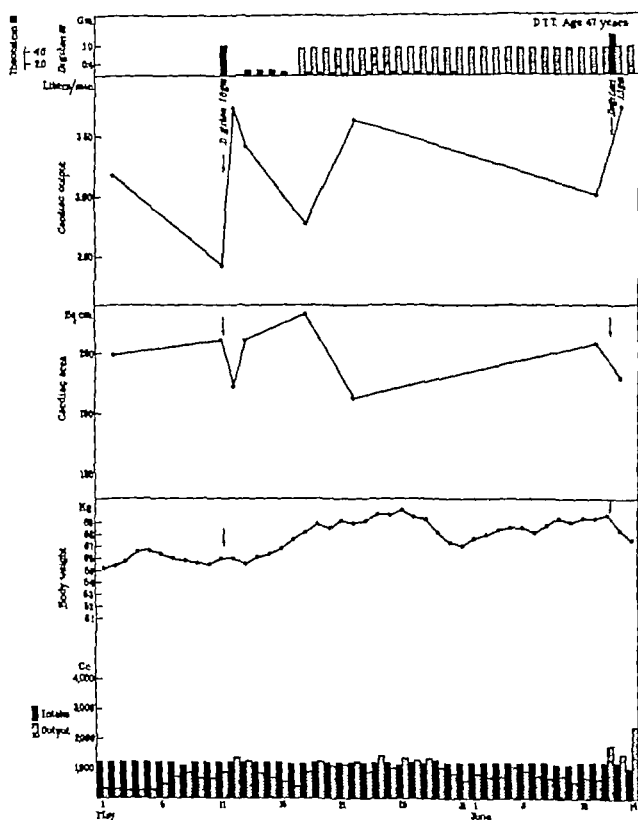
**Case 2** M. R. (Hospital number 7852), a Negro aged 33 years was admitted to the hospital on September 22, 1931. He enjoyed good health until he "caught cold" four months before admission and suffered from shortness of breath for a few days. This experience was repeated two months later. On admission he complained of shortness of breath. The heart was enlarged there were signs of mitral insufficiency and aortic roughening. There were a few râles at the bases of the lungs posteriorly. X-ray photographs of the chest showed that there was a small collection of fluid in the space between the upper and lower lobes of the right lung. The liver was enlarged. Ascites and edema of the extremities were present. The systolic blood pressure measured 150 mm Hg the diastolic 100 mm Hg. The Wassermann reaction in the blood was positive. The *etiological* diagnosis was syphilis the *anatomical* cardiac hypertrophy mitral insufficiency aortic roughening left

<sup>6</sup> In the description of events and in the figures the total cardiac output per minute is recorded. Calculation of the cardiac output on the basis of the surface area of the body (liters per minute per square meter of body surface) frequently showed the changes in a more striking manner especially if diuresis occurred and the patient lost weight. These measurements are included in the tables (Tables 3 and 4).





the liver increased in size Ten days later (October 5) the cardiac output was only 1.44 liter per minute and the heart was larger 134.2 sq cm Digitalin 1.0 gram was given a second time (October 6), but diuresis failed to occur He became however less dyspneic Next day there was a temporary rise in the cardiac output (2.21 liters per minute) and decrease in cardiac size (125.8 sq cm) He became more dyspneic the liver increased in size ascites appeared and on October 13 the output fell to 1.63 liter and cardiac size rose



B

FIG 10 A AND B IN THESE FIGURES IS REPRESENTED THE EFFECT OF GIVING DIGITALIS ON CARDIAC OUTPUT, CARDIAC SIZE AND VOLUME OF URINE IN D T T (CASE 1)

The cardiac mechanism was normal during heart failure



TABLE 3 (continued)

Case and hospital number	Age	Date	Weight	Height	Body surface	Oxygen consumption	Arterio-venous oxygen difference	Cardiac output	Cardiac output	Cardiac area	Cardiac ratio	Cardiac output	Arterial pressure	Vital capacity	Theo-calcic	Effect on T wave of electrocardiogram	Digitalis administration	Time with reference to digitalis administration
2 M.H. 7553 0	33	1931															grams	Before
		September 23	49.8	168.0	1.41	225	100.4	2.05	1.30	143.4	74	28	160/100	1500		+	1.0	24 hours after
		September 24	49.3		1.46	203	74.3	2.73	1.57	130.4	64	43	170/90	1375		+		48 hours after
		September 26	49.0		1.46	200	81.0	2.44	1.67	131.9	62	40	150/100	1325		+		
		September 29	48.4		1.42	185	68.7	2.77	1.95	132.4	70	40	160/100	1300		+		
		October 5	45.7		1.43	186	128.8	1.44	1.01	134.2	0	40	160/100	1300		±		
		October 6	46.4		1.41	197	89.1	2.21	1.57	128.8	74	30	165/100	1300		+		24 hours after
		October 7	45.4		1.41	203	101.3	2.00	1.43	131.4	76	29	165/100	1275		+		48 hours after
		October 8	45.3		1.41	203	123.0	1.64	1.16	138.6	76	23	160/100	1250		+		3 days after
		October 13	46.1		1.42	194	118.7	1.63	1.15	144.5	81	20	160/100	1125		±		
		October 14	46.0		1.42	173	71.3	2.45	1.70	138.4	72	34	145/95	1450	2.0	+		
		October 16	44.6		1.41	202	66.8	3.07	2.17	128.8	70	44	145/95	1325	4.5	+		
		October 16	43.9		1.38	197	64.3	3.04	2.20	138.2	66	44	140/90	1500	4.5	+		
		October 20	44.1		1.40	205	60.3	3.40	2.43	132.3	66	33	140/90	1700	4.5	+		
		October 27	62.5	178.0	1.78	203	122.9	2.46	1.38	248.9	116	21	120/80			+		Before
1 W.N. 7713 0	33	March 26	63.1		1.76	200	111.1	2.71	1.46	248.9	118	24	120/80			+		12 hours after
		March 29	63.3		1.79	228	104.1	2.72	1.52	257.7	123	23				+		24 hours after
		March 30	63.1		1.79	273	103.0	2.65	1.48	247.7	80	33	125/90			+		48 hours after
		March 31	63.4		1.78	260	103.0	2.45	1.41	247.7	81	31	125/90			+		3 days after
		April 1	63.4		1.78	278	109.1	2.55	1.42	247.7	83	28	120/70			±		
		April 2	63.7		1.76	276	147.7	1.57	1.04	230.7	88	21	125/70			+		3 days after



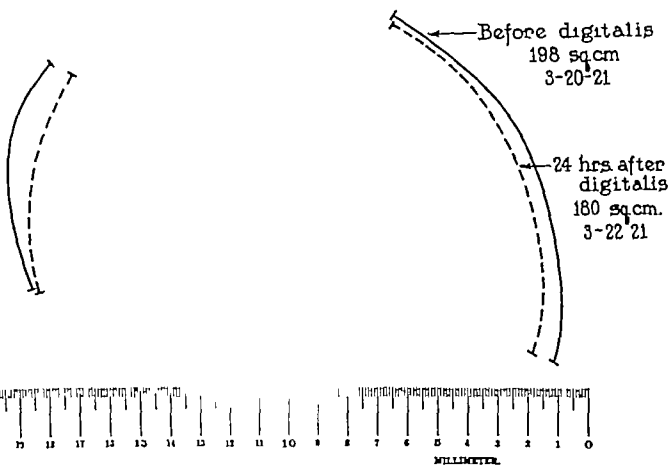
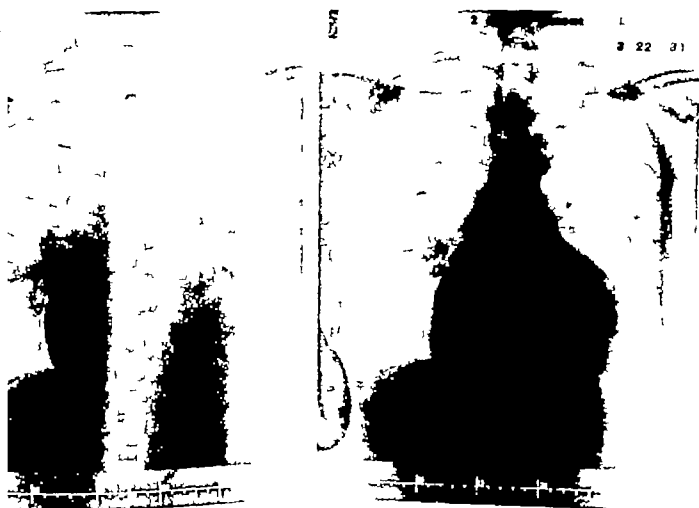


FIG 11 IN THIS FIGURE IS DEMONSTRATED THE DECREASE IN SIZE OF THE HEART WHICH OCCURRED FOLLOWING THE ADMINISTRATION OF DIGITAN 10 GRAM IN D T T (CASE 1)

The outlines of the heart were traced on thin paper from the x ray photo graphs



12 IN THIS FIGURE ARE REPRODUCED X RAY PHOTOGRAPHS OBTAINED IN THE CASE OF D T T (CASE 1)

A was taken on March 20 before and B on March 22 twenty four hours after digitalis had been given



wore off. These events were repeated to a lesser degree on a second administration of digitalis though diuresis did not occur. But when theocalcin was given, diuresis took place, the output increased and the size of the heart decreased to an amount greater than resulted from giving digitalis.

*Case 3* W N (Hospital number 7712) a white man aged 32 years was admitted to the hospital on March 26 1931. He suffered from attacks of rheumatic fever at 17, 18 and 29 years of age. His heart was affected during the first attack. Shortness of breath beginning suddenly when he was 28 years of age incapacitated him for two weeks. afterward dyspnea was present on exertion. Four months before admission dyspnea increased and edema of the extremities appeared. One month later he stopped work. Digitalis was prescribed and he became free of edema. Recently taking the drug induced ventricular premature contractions coupled rhythm resulted. He discovered that breathing deeply or coughing usually relieved him of this rhythm. On admission he was cyanotic there were a few râles at the bases of the lungs posteriorly the pleural cavities were free of fluid ascites and edema of the extremities were present and the liver was swollen. The heart was enlarged, its rhythm regular. There were signs of mitral stenosis and insufficiency and of aortic insufficiency. The *etiological* diagnosis was rheumatic fever the *anatomical* cardiac hypertrophy, aortic insufficiency mitral stenosis and insufficiency right ventricular preponderance the *physiological* regular sinus rhythm ventricular premature contractions, heart failure of the congestive type (first attack).

On March 30, the heart's output was low (2.72 liters per minute) and its size large (252.7 sq. cm.) (Table 3). On administration of digitalis (digitalin 1.0 gram) (March 30) coupled rhythm due to ventricular premature contractions occurred and persisted. Diuresis did not take place. He gained weight. Twenty four hours after taking digitalis (March 31) the cardiac output remained approximately unchanged but the size of the heart decreased slightly. Seventy two hours later, the cardiac output fell to 1.87 liter per minute. The rhythm was still coupled.

*Summary* In this instance digitalis was without diuretic effect. Although the size of the heart became smaller, cardiac output was at first unchanged, though it diminished later. Coupled rhythm occurred after digitalis was given, and persisted. Alterations in the form of the T waves of the electrocardiograms occurred.

The effect of giving digitalis when the rhythm of the heart is normal and when there are no signs of heart failure of the congestive type was shown in the case of C M (*Case 4*, Hospital number 7779) a white girl who was admitted to the hospital May 22, 1931, complaining of dyspnea. She suffered attacks of rheumatic fever at ages 5, 6, 7, 8, 9 and 12 years. When first examined at this hospital at 9 years of age, involvement of the heart had already occurred. Dyspnea first appeared when she was 13 years of age. On admission to the hospital at the age of 21 years the heart was enlarged there were signs of mitral stenosis and aortic insufficiency. Dyspnea was present. There were no signs of heart failure of the congestive type. The *etiological* diagnosis was rheumatic fever the *anatomical* cardiac hypertrophy, mitral stenosis aortic





of rheumatic fever at 26 years of age involvement of the heart occurred at that time Dyspnea and palpitation began 16 months before admission following over-exertion He took digitalis irregularly On admission the heart was enlarged Auricular fibrillation was present There were signs of mitral stenosis and insufficiency and of aortic insufficiency Cyanosis was present There were a few râles at the bases of the lungs posteriorly which disappeared within twenty four hours Edema of the extremities and ascites were not present the liver was palpable The *etiological* diagnosis was rheumatic fever the *anatomical* cardiac hypertrophy, mitral stenosis and insufficiency, aortic insufficiency, left ventricular preponderance, the *physiological* auricular fibrillation

When admitted to the hospital he was under the influence of digitalis the ventricular rate was slow, the pulse deficit small On November 11, the cardiac output measured 2.58 liters per minute, the cardiac size, 154.9 sq cm, and the vital capacity, 2200 cc. (Table 4, Figure 15) As the effect of digitalis wore off the ventricular rate became more rapid and the pulse deficit wider Body weight increased and dyspnea became more severe He complained of palpitation and cardiac pain The liver became larger On November 28 the cardiac output fell to 2.23 liters per minute, the size of the heart rose to 159.7 sq cm and the vital capacity fell to 1600 cc On the administration of digitalis in the form of digitan 1.0 gram after measurements were made on November 28, diuresis occurred promptly, the ventricular rate became slower and the patient felt better Twenty four hours later his output increased to 3.40 liters per minute, the size of the heart fell to 153.6 sq cm, and the vital capacity rose to 1800 cc The administration of digitalis was continued until December 4 Diuresis continued, he lost weight and became free of the signs and symptoms of heart failure As before, when the drug was no longer given, its effect wore off Heart failure (cyanosis, dyspnea, palpitation, cardiac pain enlargement of the liver, rapid ventricular rate, gain in body weight, decrease in vital capacity) reappeared The cardiac output fell to 1.67 liter per minute (January 5) and the size of the heart increased to 164.6 sq cm Venous pressure measured 11.6 cm of blood Digitan 1.0 gram was given a second time Diuresis again occurred promptly and the ventricular rate again became slow The output increased to 2.47 liters per minute and the size of the heart fell to 144.5 sq cm The venous pressure fell to 3.0 cm of blood and the vital capacity increased to 1900 cc Diuresis continued until the patient became free of heart failure

**Summary** In this case the volume output was less and the size greater during heart failure than during the state of compensation On administration of digitalis, cardiac output increased, cardiac size diminished, vital capacity increased, venous pressure decreased, ventricular rate became slower, and the patient became free of the signs and symptoms of heart failure As the effect of digitalis wore off, changes occurred in the reverse direction

**Case 8** M C (Hospital number 7489), a woman 51 years of age was admitted to the hospital on May 6, 1931 Dyspnea appeared first when she was 46 years of age A first attack of heart failure occurred two years later On admission she was suffering from a fourth attack of heart failure. She had been complaining of shortness of breath and swelling of the feet and

TABLE 3

*Effect of giving digitalis on cardiac output, cardiac size, vital capacity, and venous pressure in patients exhibiting auricular fibrillation and heart failure*

Case and hospital number	Age	Date	Weight lbm	Height cm	Body surface sq m	Oxygen consumption cc per minute	Aortic-venous oxygen difference cc	Cardiac output liters per minute	Cardiac area sq cm	Cardiac rate min/100	Venous pressure mm Hg	Vital capacity cc	Effect on T wave of electrocardiogram	Dose in grams	Time before or after digitalis
1 H.C. 7462 ♂	31	1931													
		November 11*	61.0	163.5	1.65	242	81.0	2.58	151.0	61		2700	-T <sub>1</sub> ±T <sub>2</sub>		
		November 28	67.9		1.70	245	109.5	2.23	159.7	100		1600	T <sub>1</sub> less neg. ±T <sub>2</sub>		
		November 29				255	75.0	3.10	153.6	68		1800	T <sub>1</sub> more neg. ±T <sub>2</sub>	1.0	1 hr. after
		November 30	62.9		1.65	210	95.0	2.50	156.2	68		1700			
		December 1	61.4		1.64	215	91.0	2.67	157.7	65		1400			
		December 3	59.3		1.66	213	89.7	2.37	155.4	67		1900		0.5	1 hr. after
		December 10	56.8		1.63	222	89.0	2.50	160.8	66		2200		0.5 (over 1 hr.)	1 hr. after
		1932											Change in reversed position		
		January 5	59.4		1.64	218	130.7	1.67	164.0	100		1700	T <sub>1</sub> less neg. ±T <sub>2</sub>	1.0	1 hr. after
6 M.C. 7469 ♀	51	1931													
		May 20	59.0	163.0	1.63	109	81.5	2.44	185.3	91†		1650	+T <sub>1</sub> ±T <sub>2</sub>	1.0	Before
		May 20				208	74.6	2.70	170.5	56		1800	±T <sub>1</sub> -T <sub>2</sub>		24 hr. after
		May 21	58.1		1.62	214	62.0	3.45	167.3	60		1825	±T <sub>1</sub> -T <sub>2</sub>		18 hr. after
		May 22	56.0		1.61	212	66.0	3.21	167.4	82		1710	±T <sub>1</sub> -T <sub>2</sub>		
		May 27	50.4		1.60	212									
		1933													
		January 10	81.3	161.0	1.89	281	188.9	1.78	109	†		1600	+T <sub>2</sub>	0.5	Before
		January 20	83.1		1.88	275	110.8	2.48	†	70		1600	-T <sub>1</sub> ±T <sub>2</sub>		24 hr. after
		January 21													
7 M.J. 5783 ♀	38	1933													
		January 10	82.3		1.87	306	75.5	4.05	†	81		1500	±T <sub>1</sub> ±T <sub>2</sub>		
		January 22	77.0		1.82	295	89.2	3.31	†	96		1700			
		January 30													

\* Patient was under the influence of digitalis on admission to hospital

† The radial pulse rate was counted in this patient

‡ The size of the heart could not be measured, see text

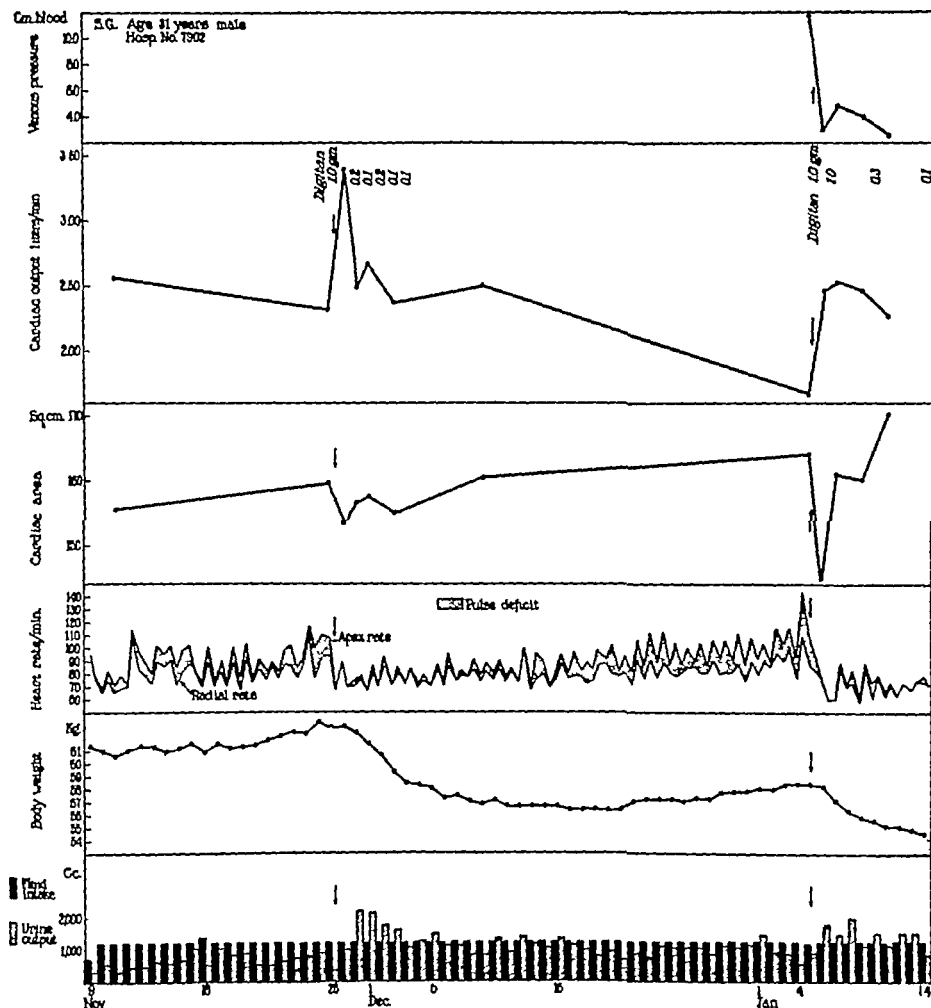


FIG 15 IN THIS FIGURE IS REPRESENTED THE EFFECT OF GIVING DIGITALIS ON CARDIAC OUTPUT CARDIAC SIZE AND VOLUME OF URINE IN S G (CASE 5), THE SUBJECT OF AURICULAR FIBRILLATION

abdomen for four weeks. The *etiological* diagnosis was unknown, the *cardiac* enlargement of heart, chronic myocarditis, right ventricular preponderance, mitral insufficiency, the *physiological* auricular fibrillation, ventricular premature contractions, heart failure of the congestive type (fourth attack).

The cardiac output was 2.44 liters per minute on May 20, and the size of the heart 185.3 sq cm (Table 4, Figure 16). She had not taken digitalis

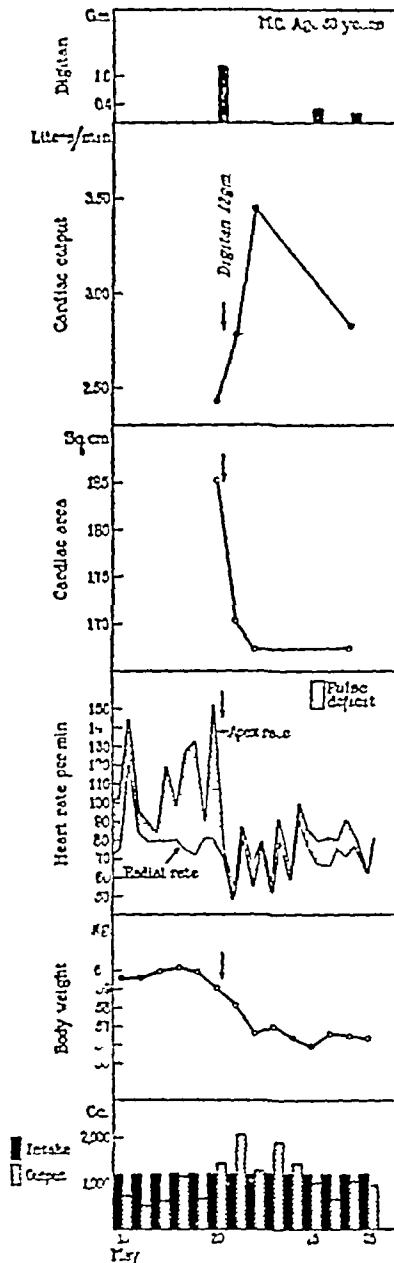


FIG 16 IN THIS FIGURE IS REPRESENTED THE EFFECT OF GIVING DIGITALIS ON CARDIAC OUTPUT, CARDIAC SIZE AND VOLUME OF URINE IN M C (CASE 6) EXHIBITING AURICULAR FIBRILLATION

since admission to the hospital. The ventricular rate was rapid, the pulse deficit was great, there were a few râles at the bases of both lungs posteriorly. There was edema of the extremities. Ascites was present, the liver was enlarged. On the same day she was given digitalis (digitalin 1.0 gram). The ventricular rate became slower, diuresis occurred, she lost weight, râles, edema and ascites disappeared, and the liver became smaller. Two days later (May 22) the cardiac output rose to 3.45 liters per minute and the size of the heart fell to 167.3 sq. cm. The vital capacity rose from 1650 to 1825 cc.

**Summary** In this patient exhibiting auricular fibrillation, the cardiac output was diminished and the size of the heart large during heart failure. On the administration of digitalis the output rose, the size diminished and the patient became free of edema.

**Case 7** M. J. (Hospital number 5783), a white woman, 38 years of age, was admitted to the hospital on January 11, 1932. Hypertension had been present for five years and auricular fibrillation for three years. On admission, suffering from a seventh attack of heart failure, she exhibited marked edema of the extremities and enlargement of the liver. There were a few râles at the bases of the lungs posteriorly. The heart was large, the ventricular rate rapid. The systolic blood pressure measured 180 mm. Hg, the diastolic 140 mm. Hg. The *etiological* diagnosis was arterial hypertension, the *anatomical* enlargement of heart, left ventricular preponderance, the *physiological* auricular fibrillation, intraventricular heart block, heart failure of the congestive type.

The cardiac output measured 1.78 liter per minute on January 19, and the venous pressure, 9.5 cm. blood (Table 4, Figure 17). The heart was enlarged to such an extent that its left border in the x-ray photographs could not be distinguished from the shadow of the chest wall. For this reason its size was not measured. Digitalis (digitalin 0.8 gram) was given, the ventricular rate decreased and 24 hours later (January 20) the cardiac output rose to 2.48 liters per minute, and the venous pressure fell to 6.5 cm. blood. As diuresis did not occur at once, the administration of theocalcin was begun on January 21. Cardiac output increased to 4.05 liters per minute and the venous pressure fell to 3.5 cm. of blood. Diuresis occurred and persisted until the patient became free of edema.

**Summary** In this patient exhibiting auricular fibrillation with a rapid ventricular rate, the cardiac output was diminished during heart failure. Following the administration of digitalis, output increased, venous pressure fell, and ventricular rate became slower. The administration of theocalcin was more effective than digitalis in the relief of heart failure. It resulted also in greater increase in cardiac output.

#### SUMMARY

The experience gained from a study of these patients makes it clear that (1) the volume output of blood per minute from the heart in human beings is diminished during heart failure, both when the cardiac mechanism is normal and when auricular fibrillation is present.

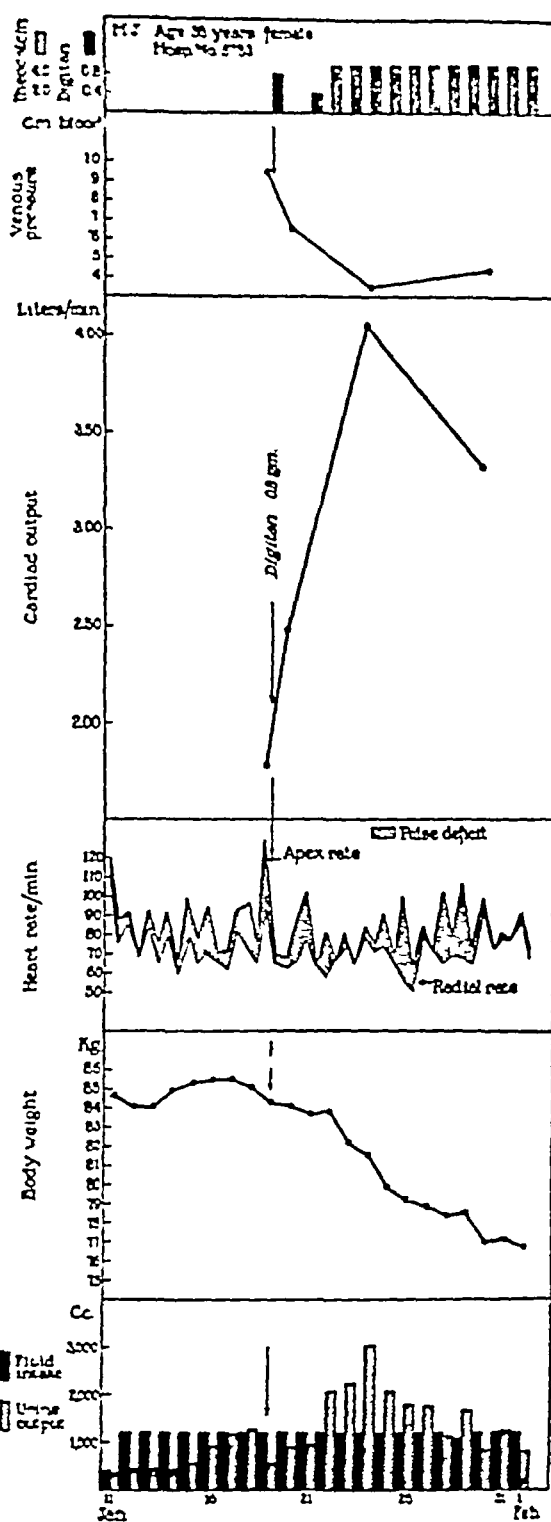


FIG 17 IN THIS FIGURE IS SHOWN THE EFFECT OF GIVING DIGITALIS AND THEOCALCIN ON CARDIAC OUTPUT, CARDIAC SIZE AND OUTPUT OF URINE IN M. J. (CASE 7), THE SUBJECT OF AURICULAR FIBRILLATION

(2) During heart failure the size of the heart is larger than it is when in a state of compensation

(3) When improvement results, the administration of digitalis occasions (a) *increase* in cardiac output, (b) *decrease* in cardiac size, (c) *decrease* in venous pressure, (d) *increase* in vital capacity, (e) *slowing* of the ventricular heart rate (f) Changes in the form of the electrocardiogram are observed at this time

(4) The administration of theocalcin was more effective in the relief of heart failure than digitalis in two patients. Giving it occasioned (a) *increase* in cardiac output, greater in amount than resulted from giving digitalis, (b) *decrease* in cardiac size, and (c) *decrease* in venous pressure

#### DISCUSSION

In all subjects, normal as well as those suffering from heart failure, alterations in form of the *T*-waves of the electrocardiograms followed the administration of digitalis. They were frequently the earliest effects observed. The alterations were not uniform, the most characteristic and usual one was *negativity* of  $T_1$  (21), in certain cases  $T_1$  and  $T_2$ , however, also became negative,  $T_1$  or  $T_2$  or  $T_3$  sometimes became diphasic. On the other hand, waves which were negative became positive, and finally changes in amplitude only, occurred. The point to be emphasized is that *change* in form of the waves *always* took place.

There is ample experience with theocalcin in the treatment of heart failure to show that it is often useful in the relief of edema when digitalis fails (22). The new experience, although limited to observations in two patients, supplies evidence that in addition its action is accompanied by increase in cardiac output and decrease in cardiac size and in venous pressure. What the mechanism is by which theocalcin accomplishes these results is still unknown. Various possibilities suggest themselves, a xanthin effect on the rate of coronary flow, a calcium ion effect on heart muscle, or still another form of calcium action.

It has already been shown in Part I of this paper that the results of the administration of digitalis to normal individuals differ in several respects from those just described in patients. These differences include (a) *decrease* in cardiac output, (b) *decrease* in cardiac size, (c) no significant alteration in the level of venous blood pressure, (d) no change in vital capacity.

A matter of major importance which has emerged from conducting these researches is the paradox that one and the same drug, digitalis, when given to human beings has, in normal persons, one effect, and in persons the subjects of heart disease, another, decrease in the output of blood in the former, increase in the latter, as Burwell, Neighbors and Regen found (1). In parallel situations in dogs (normal (23) and pathological (24)) the results resemble those now reported. Is the difference in



result due to an essential difference in action or is it due to a difference in the essential conditions which are being investigated?

In both normal and diseased hearts, in human beings and in dogs the action of digitalis has, at least in one respect, an identical consequence, in both, the size of the heart becomes smaller. It has also another consequence, the vigor of its action increases. If this consequence were not known from former experience (Cohn and Stewart (24, 25, 26)) it would have become apparent from the present ones in which the output of blood in diseased persons increases. The difference in the effect in the two types of heart, normal and pathological, results, so we think and as we have formerly stated, from the fact that normal hearts when under its influence become smaller but, being smaller pumps, expel smaller volumes, though contraction is more effective. The net result of its action, if it depends upon an effect on the hepatic veins, does so to a minor, perhaps to a negligible, degree only. Pathologically enlarged hearts likewise become smaller, but not so small that their cavities shrink to dimensions less than normal, in point of fact, though they decrease, their final dimensions are larger perhaps than when they were in their healthy state. They attain, due to the action of digitalis, sizes commensurate with ejecting larger volumes than were possible in the state of failure. Whether decrease in size to the extent to which it occurs facilitates the result, the expulsion, namely in heart failure, of larger quantities than before, or whether the increase in contractile capability brings it about, the fact is nevertheless clear that the heart has become smaller and that the circulation which was insufficient before, has become sufficient. The evidence for improvement is to be found in the amelioration of the symptoms and in changes in the other physical signs.

The explanation of these events may perhaps be found in Starling's theory dealing with the length of the muscle fibers of the heart (27). The theory is too well known to require repetition. It is not a wide stretch to believe that it is applicable in these cases in which there is such clear evidence of change in size of the heart and of increase in its functional capacity.

There is required still, consideration of that aspect of the problem which Dock and Tainter (3, 4) have brought forward. The major part of the argument which deals with their contention and the evidence which leads us to take a different view, is contained in a former paper (28). It is necessary to discuss one more phase of the matter. In cases where the output becomes smaller, as in the normal heart, the point has been made (3, 4) that this is due, not to a change in diastolic size of the heart, a change in tone, but to decrease in inflow, a result of constriction of the hepatic veins, indicated by the fact that the venous pressure is low. Low venous pressure is, on the latter assumption, regarded as the sign of

a small volume of blood available for inflow into the heart. Low venous pressure was found by Dock and Tainter in dogs, which they studied, for a maximum of two hours after the injection of a digitalis substance.

The plain result of the measurement of the venous pressure in normal human beings is that a change in pressure parallel with the volume of the output does not occur. Based on these observations, and in view of the anatomical arrangement in the liver of human beings, the mechanism proposed by Dock and Tainter seems untenable. A reason for the difference in interpretation can be given to the last statement, depending on a difference in the times when their observations and ours were made—theirs after an interval varying from a few minutes to two hours, ours after many hours and as a matter of fact after several days. The duration of their observations was, as has been pointed out, brief. If the course of the entire subsequent action in dogs is determined by events in these early minutes, we have no counter argument to offer. But it seems unlikely in healthy human beings that, if the outflow is small and the venous pressure normal or even elevated, as in several instances, the level of the venous pressure can be responsible for the result, in the sense in which Dock and Tainter wish to attribute to it a deciding influence. The venous pressure, far from being low, is too inconstant in its behavior to bear the burden of responsibility for so conspicuous an action as the decrease in output. What the venous pressure is in healthy intact dogs, after hours or days, is still unknown. Information on this point is desirable.

On the other hand we have demonstrated again, and it is an observation which is now well known, that fall in venous pressure is not an infrequent occurrence following the administration of digitalis to patients suffering from heart failure and edema. Increase in cardiac output occurs in such cases, as these observations show, at a time when venous pressure falls, venous pressure, in short, is high when the output is small and falls as output increases. The venous pressure is high under these circumstances not because the venous return is great but because the heart is incapable of propelling through its cavities all the blood which comes to it.

It seems necessary, therefore, in the case of normal hearts, the evidence being what it is, to hold belief in the theory that decrease in outflow is due to increase, expressed as decrease beyond the natural, perhaps optimal length, in tone of the heart muscle.

#### CONCLUSIONS

- 1 A consequence of the action of digitalis is to decrease the volume output of blood per minute from the heart in normal human beings and to decrease its size.

- 2 The volume output of blood per minute from the heart which is in failure is diminished and its size larger than when it is in a state of compensation.

3 Following the administration of theocalcin in patients during heart failure, cardiac output increases and cardiac size and venous pressure diminish

4 Giving digitalis increases the volume output of blood per minute from failing hearts and decreases their size

5 Digitalis, we think, has similar, perhaps identical, actions both in normal and in diseased hearts, it *decreases* cardiac size and *increases* the extent of ventricular contraction (23) The consequence of these actions is that the volume of the cardiac output which results differs, depending on an initial difference in size of the ventricular cavities, in the two situations In the one, the normal heart, it becomes too small, in the other, the diseased heart, it develops a suitable size

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# THE LACTIC ACID OF THE BLOOD IN HEPATIC DISEASE<sup>1</sup>

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## LITERATURE

The relation of the liver to the metabolism of carbohydrates has attracted the attention of investigators since Minkowski (1886) demonstrated a decrease in blood sugar and an increase of excretion of lactic acid in the urine of hepatectomized geese. The function of the liver in the maintenance of the normal value for blood sugar is now fully established (Mann and Magath) and attention naturally turns to the intermediate products of metabolism of carbohydrate such as lactic acid. Von Noorden and Embden (1906) first advanced the idea of a carbohydrate cycle between liver and muscle and suggested that the lactic acid formed by muscular contraction was returned to the liver to be stored as glycogen. This view has been fully confirmed by recent investigators, notably by Himwich and his collaborators and by Cori and Cori. It is now recognized that in the normal resting organism some lactic acid passes from the muscles to the blood, and small amounts (7 to 18 mgm. each hour) appear in the urine. Much larger amounts of this substance appear in both blood and urine after exercise; the quantity is affected considerably by the degree of "oxygen debt." The liver undoubtedly removes a considerable part of this excess lactic acid from the circulation and stores it as glycogen. It has been shown (Schneider and Widmann) that the liver is capable of retaining considerable amounts of optically inactive lactic acid injected into the portal vein; the concentration of lactic acid of the hepatic venous blood is not increased, nor is its optical activity increased.

From the foregoing, it is evident that the functional efficiency of the hepatic parenchyma must have a considerable effect on the value for lactic acid in the blood, and on the rate with which the accumulations of this substance are removed following exercise. As the recent experiments of Eggleton and Evans disclosed, the liver is not the only agent in removing lactic acid from the blood. The heart and brain take up this substance readily and resting muscles absorb some of the lactic acid liberated by working muscles. Considerable amounts may also be lost in the perspiration.

Muscular exercise is, of course, the factor most likely to produce an increase in lactic acid of the blood in the individual case. Other factors also elevate the value for lactic acid of the blood; among these may be mentioned cardiac decompensation with circulatory failure (Meakins and Long), anemia, anoxemia, prolonged vomiting, irradiation, ether narcosis (Ronconi and coworkers), and administration of various sugars (Campbell and Maltby, Wierzychowski

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and Langerhans). The presence of carcinoma is also said to increase the concentration of lactic acid in the blood (Büttner, Valentin, Schumacher, Geelen and Frankstein). In general, the value for lactic acid of the blood is highly variable, and all of the factors mentioned must be taken into consideration when one attempts to evaluate the individual test. The general significance of lactic acid in the organism, and the factors which influence its metabolism in health and disease, recently have been considered in detail by Humwich, by Jervell and by Cori and need not be considered in further detail here.

A number of papers (1, 2, 3, 4, 6, 10, 14, 20, 21, 23, 28, 29, 33, 34, 35, 36) on the value for lactic acid in the blood in cases of hepatic disease and in experimental hepatic injury have appeared in the last few years. Although there are variations in the methods used and in the results obtained, most investigators agree that in the more severe degrees of hepatic injury, there is an increase in the lactic acid of the blood. This increase may be slight and inconstant in association with minor degrees of hepatic injury, and is often small even in severe cases. It has also been shown that there is delay in the disappearance of lactic acid from the circulation following injection of lactates into patients with hepatic disease and in animals with experimental lesions of the liver. Again, widely varying amounts of lactate have been given by various authors, and the results are not altogether in agreement. The recent studies of Bollman and Mann, of the metabolism of lactic acid following hepatectomy performed on dogs, and similar studies by Drury and McMaster on hepatectomized rabbits, constitute the principal reason for reopening the subject at the present time. Briefly, these workers have found that removal of the liver is followed by increase in the lactic acid content of the blood and urine. The initial increase, which usually reaches a value about double the normal amount, is probably due chiefly to ether anesthesia, since the value for lactic acid of the blood falls during the period of recovery. A somewhat greater terminal increase is also noted in the majority of animals just before death. Neither development of hypoglycemia nor injection of glucose appears to affect the value for lactic acid in the blood after hepatectomy (Bollman and Mann). Bollman and Mann showed that following intravenous injection of lactic acid or sodium lactate into these liverless animals, the value for lactic acid of the blood increases sharply. Only a small portion of this injected lactic acid (approximately 20 per cent) is excreted in the urine, and the amount present in the blood returns to its previous level in a short period. These changes in lactic acid of the blood noted after hepatectomy give a standard of comparison for the study of this subject in clinical cases of hepatic disease, and furnish some idea of what might be expected in clinical conditions of hepatic insufficiency.

#### MATERIAL AND METHODS

The present study consists of 250 determinations of lactic acid of the blood. The subjects studied included normal persons under varying conditions of exercise and activity and patients with hepatic disorders of various types, eighty in all. In certain cases it was possible to make repeated determinations of lactic acid at various stages of hepatic disease, and thus to study any relationship that may exist between the clinical condition of the patient and the lactic acid content of the blood. Determinations of lactic acid were also made before and after surgical operation

for relief of obstructive jaundice due to stone in the common bile duct, to carcinoma, and to stricture. The values for lactic acid of the blood were also determined in a small group of cases following intravenous injection of solutions of sodium lactate.

Lactic acid was determined by the method of Friedemann and Kendall. With persons under basal conditions, the normal value for lactic acid, as determined by this method, lies between 8 and 15 mgm in each 100 cc. of blood. Even slight muscular exertion increased this value considerably, but in general it was found that twenty to thirty minutes in the prone position following moderate degrees of muscular activity, was sufficient to restore to normal the proportion of lactic acid in the blood. It is obvious, of course, that after more prolonged and vigorous activity, a longer period is required before the basal level is reached. The use of a tourniquet was avoided whenever possible, but in our experience the very brief period of venous stasis required to obtain a sample of blood had little, if any, effect on the result obtained. This has also been the experience of Hewlett, Barnett and Lewis. Prolonged venous stasis may produce a considerable elevation of the value for lactic acid in the blood (Mendel and his coworkers), but it is rarely if ever necessary to cause prolonged venous stasis in obtaining specimens of blood.

In general it was found that hepatic disease produced slight to moderate elevations in the value for lactic acid in the blood. The greatest rises were noted among patients who gave clinical evidence of severe intrahepatic types of jaundice, and among patients with carcinomatous obstruction of the biliary passages. The data on a representative group of these cases are presented graphically in Figure 1. The additional cases gave results in accord with these figures.

The findings are in accord with those of other observers, and no doubt the changes noted represent interference with normal recovery of lactic acid by the liver. The increases noted are fully as great as those observed in work with many animals after hepatectomy, this will be considered later. It is of interest to note that the values for lactic acid in the blood rarely exceed the renal threshold of 30 to 40 mgm for each 100 cc of blood as established by Hewlett, Barnett and Lewis. Apparently renal elimination constitutes a check on the accumulation of lactic acid in the blood, and further studies of urinary excretion of this substance are being made in order to corroborate this hypothesis.

Loeb and his coworkers stated that there is no demonstrable relationship between either the clinical condition of the patient or the degree of bilirubinemia and the observed values for lactic acid of the blood. An attempt at correlation between the values for serum bilirubin and those for lactic acid is illustrated graphically in Figure 2. It is obvious that no close correlation exists, the actual correlation coefficient is calculated as 0.1. However, these data appear to show that increases in serum



bilirubin are consistently associated with values for lactic acid which are above normal. It is evident that no very close correlation can be expected among patients with obstructive jaundice, since the value for bilirubin among these patients may fluctuate considerably from day to

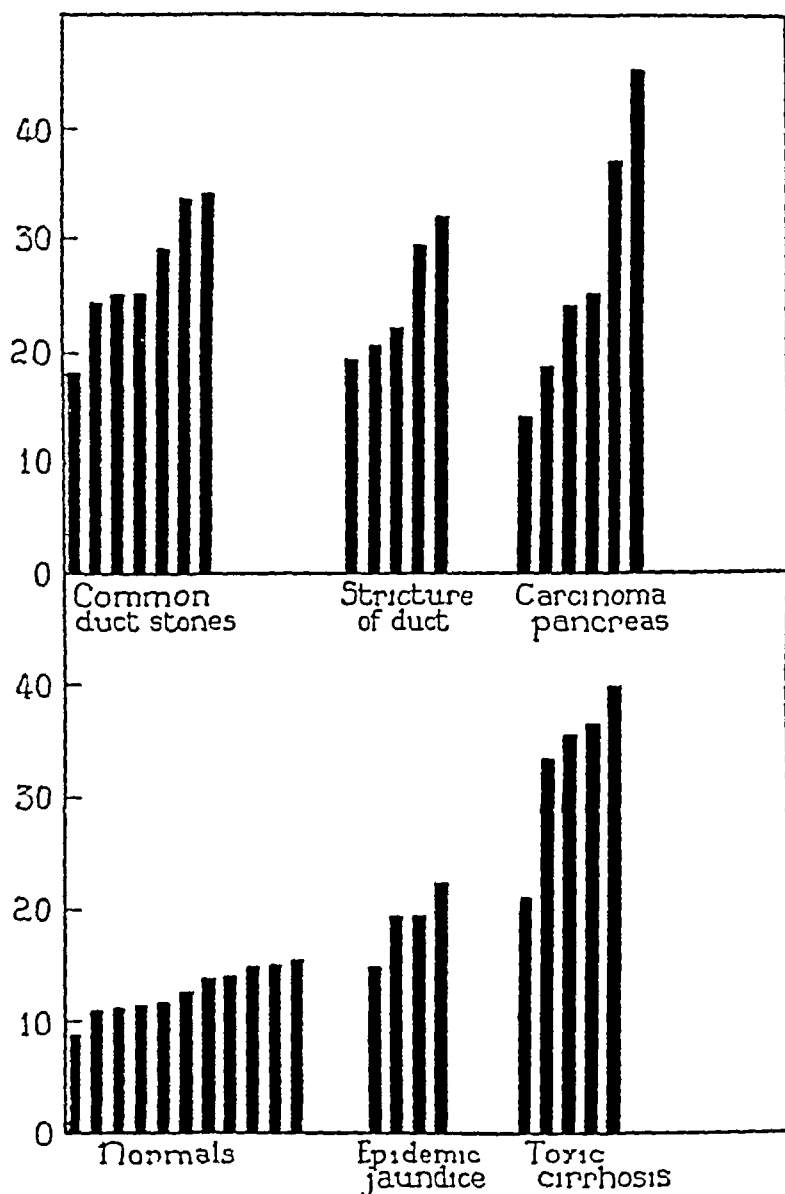


FIG. 1 THE CONTENT OF LACTIC ACID OF BLOOD IN NORMAL PERSONS AND IN PATIENTS WITH VARIOUS TYPES OF HEPATIC DISEASE

day, and may be affected by many factors not necessarily connected with the functional efficiency of the hepatic cell. In jaundice due primarily to hepatic parenchymal injury, a closer correlation may exist. In one case

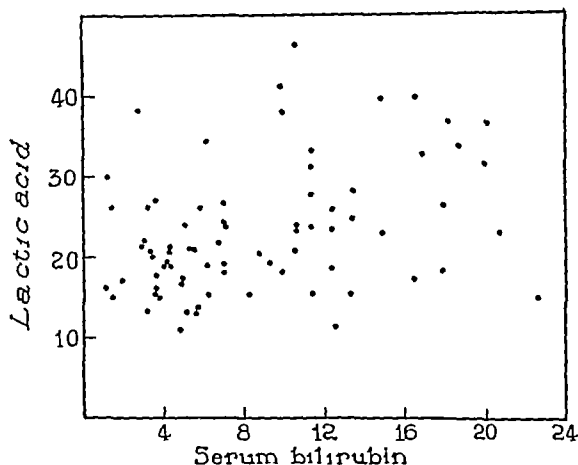


FIG 2 THE RELATION OF SERUM BILIRUBIN AND LACTIC ACID IN THE BLOOD IN JAUNDICED PATIENTS

of hepatitis due to arsphenamine, the values for lactic acid and bilirubin during the stage of recovery paralleled each other closely (Fig 3) In one case of infectious jaundice, similar correlation was noted

It is recognized that in mechanical obstruction to the bile passages

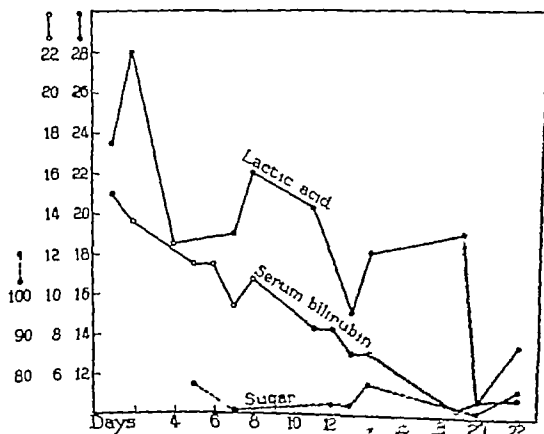


FIG 3 VALUES FOR THE SERUM BILIRUBIN AND LACTIC ACID IN THE BLOOD IN A PATIENT WITH ARSPHENAMINE HEPATITIS

the general degree of hepatic parenchymal injury occurs. In favorable cases it is probably repaired rather promptly following relief of biliary obstruction. It is also known that the glycogen content of the liver is greatly decreased in the presence of mechanical obstruction to the biliary passages, and that it returns to normal when the obstruction is relieved. Under basal conditions the values for lactic acid of the blood, following surgical relief of biliary obstruction, vary in a way that may be correlated, in general, with these changes (Table 1). In favorable cases there is a

TABLE 1

*Values for lactic acid and other constituents of the blood before and after relief of biliary obstruction*

Remarks	Date	Constituents			
		Blood			Serum
		Lactic acid	Sugar	Urea	Bilirubin
	1932	mgm per 100 cc	mgm per 100 cc	mgm per 100 cc	mgm per 100 cc
Cholelithiasis, diabetes mellitus	February 26	24.9	162	20	4.1
Following biliary colic	February 27				9.4
Second biliary colic	March 1	41.1	207		10.0
Cholelithiasis	March 3				
	March 7	21.2	117		5.4
	March 10	23.9	110	26	5.2
	March 14	21.9	101		5.8
	March 17	20.1			4.7
	March 22	10.8	87		4.7
	March 24	12.6			4.3
Patient up and about	March 28	22.1			3.2
	April 1	22.1	97		2.5

temporary rise of lactic acid in the blood following operation, due probably to the effect of anesthesia. This is followed by a period of decline until a resting normal level is established. As soon as the patient is permitted to be up and about, however, a rather constant rise in the value for lactic acid is noted. This rise was observed in several cases, and can be attributed to factors involving muscular and circulatory efficiency rather than to the liver. In cases in which failure to recover follows relief of biliary obstruction, the value for lactic acid increases rather than declines, and may reach high levels as the clinical condition of "hepatic insufficiency" develops. Data in a group of such cases are presented in Table 2. It will be noted that very high levels for lactic acid and the

TABLE 2  
*Values for lactic acid and other constituents of the blood in cases in which recovery did not follow relief of biliary obstruction*

Case	Age (years) and sex	Diagnosis	Deter- mina- tion made days before death	Lactic acid per 100 cc. mean	Urea, per 100 cc. mean	Bilirubin, of serum mgm. per 100 cc.	Intake of fluid	Output of urine	Comment
1	36F	Stricture of common bile duct, nod- ular cirrhosis of liver, ascites	5	51.3		7.7	950	150	Impending hepatic coma
2	54F	Cholelithiasis portal cirrhosis (cho- lecystectomy)	2	219.0	64	6.0	2600	500	Alternating periods of coma and vio- lent muscular activity
3	57M	Carcinoma of duodenum (cholecys- tostomy)	7 6	75.8 54.8	102 142	6.3 8.6	2980 3350	1025 170	Drowsiness Impending coma suppression of bile and urine In extremis
4	62M	Stone in common bile duct (choledo- cholotomy)	2 13 5	94.1 39.0 58.0	225 98 66	7.9 16.3 4.7	5300 3750 2600	400 1490 300	Drowsiness restlessness muscular twitching Great weakness failing urinary out- put irrationality
5	67M	Carcinoma of common bile duct (cholecystostomy)	6 5	39.8 52.4		16.7 18.3	3000 3300	1100 1700	Temperature 100.5° F very poor biliary drainage Increasing weakness and apathy
6	69M	Carcinoma of head of pancreas (cholecystostomy)	1	58.7	93	25.0	3800	Unknown	Periods of stupor alternating with muscular activity
7	44M	Renal and hepatic amyloidosis	10 3	22.0 33.5	156 336	4.3 7.6	1500 2100	600 425	Extreme emaciation and muscular wasting Deep coma
8	71M	Carcinoma of pancreas (cholecysto- gastrostomy)	4	31.2	158	19.2	1840	1300	Asthenia deep stupor



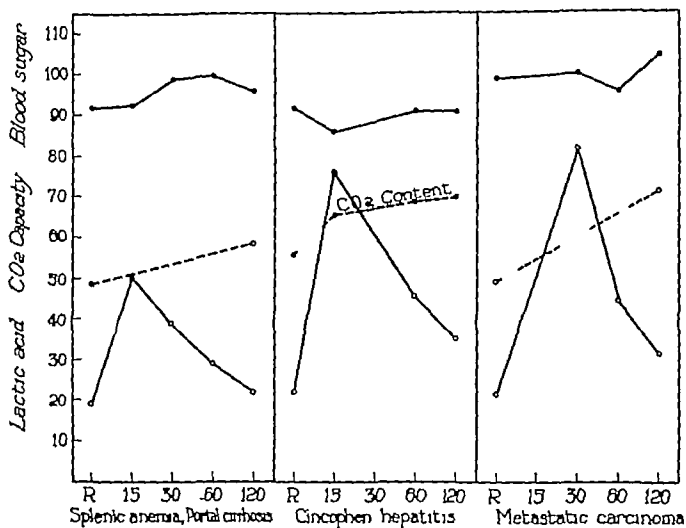


FIG 4 THE UTILIZATION OF SODIUM  $\gamma$  LACTATE IN VARIOUS TYPES OF HEPATIC DISEASE

presented graphically in Figures 4 and 5. The value for lactic acid of the blood of one patient who had almost completely recovered from jaundice caused by *arsphenamine* promptly declined following administration of sodium lactate, and in general gave a normal result. There was definite delay when compared with the normal figures given by Hartmann and Senn in disappearance of lactic acid from the blood following the injection, of one patient with metastatic carcinoma of the liver and one with splenic anemia and portal cirrhosis. Neither of these patients was jaundiced, but retention of bromsulphalein was graded 2 and 3, respectively. A third patient who was recovering from the effects of toxic hepatitis due to cinchophen, also retained injected sodium lactate to some degree. The degree of retention of lactic acid in each case corresponded closely with the clinical conception of the degree of hepatic injury present in the case, and in some instances also corresponded with the results either of the galactose or bromsulphalein tests for hepatic function (Table 3). Judging from the value for blood sugar in these cases, it is apparent that the lactic acid injected was either stored as glycogen in the liver or was utilized in some other fashion. As has previously been stated, similar disappearance of injected sodium lactate has been noted in work with hepatectomized animals.

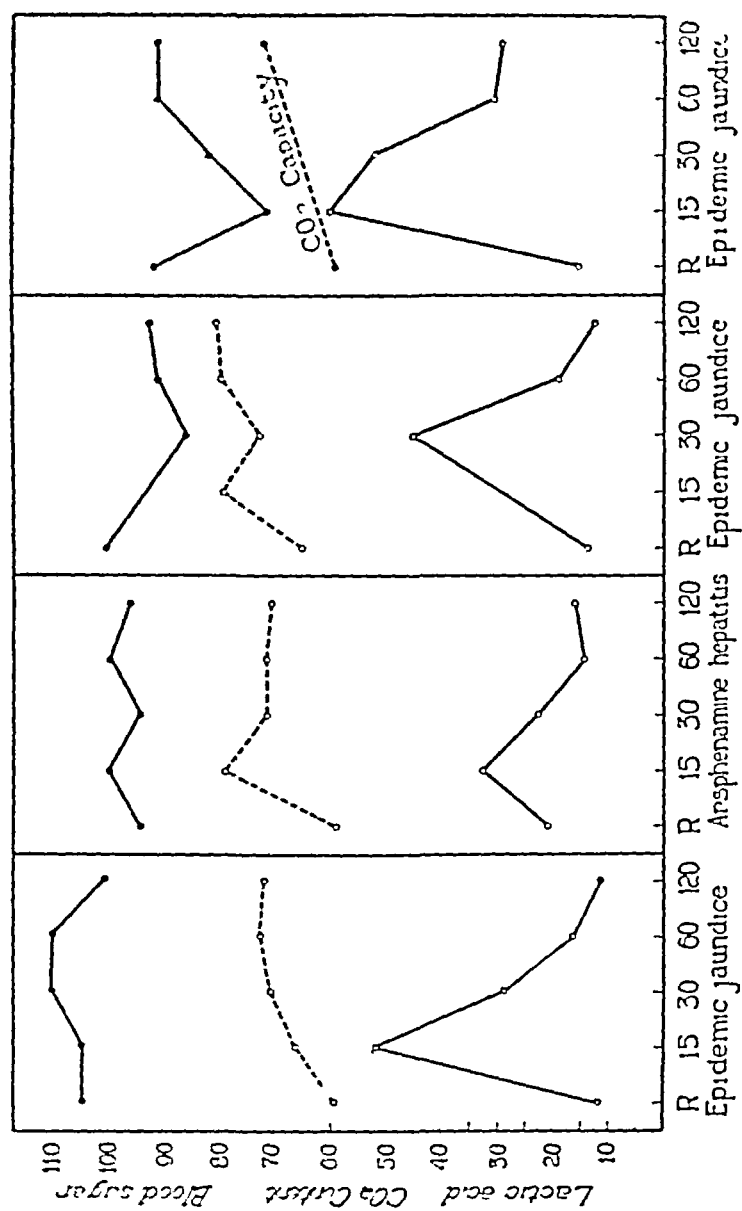


FIG. 5 THE UTILIZATION OF SODIUM *r*-LACTATE IN VARIOUS TYPES OF HEPATIC DISEASE

TABLE 3

*Comparison of utilization of sodium lactate with results of bromsulphalein and galactose tests of hepatic function*

Diagnosis	Sodium <i>r</i> lactate metabolism test, 4.5 cc. of molar solution given intravenously for each kgm. of body weight			Bilirubin of serum	Retention of bromsulphalein grade	Excretion of p-lactate
	Resting value	Maximal rise	Value at end of two hours			
	mgm.	mgm	mgm	mgm per 100 cc		grams
Epidemic jaundice	15.3	60.9	28.9	4.3		4.5
Epidemic jaundice	14.4	45.5	13.1	5.2		3.4
Epidemic jaundice	12.2	53.0	12.2	25.0		3.7
Toxic cirrhosis	13.8*	26.3	12.5	13.6		5.0
Hepatitis from arsphenamine	21.6	32.9	15.7	3.1		1.3
Hepatitis from cinchophen	22.5	76.2	35.6	5.4		3.0
Splenic anemia	18.9†	50.0	23.0	2.0†	3	
Metastatic carcinoma of liver	22.1	83.4	32.5	§	2	

\* 10 grams of sodium *r* lactate

† 2.25 cc. of molar sodium *r* lactate for each kgm. body weight

‡ Indirect

§ Indirect, less than 1.0

## COMMENT

The increased values for lactic acid of the blood which we have obtained among patients with various types of hepatic disease are in general corroborative of those given by previous investigators of the subject. The average increases noted were somewhat greater than those previously reported, a fact which may be explained on the basis of differences in available clinical material and in the methods used to determine lactic acid. In general, our figures agree closely with those recently reported by Diaz and Cuenca, and by Massobrio and Micznisoff.

We were able satisfactorily to exclude the effects of cardiac, venous stasis, circulatory or respiratory disorders, and anemia in the cases under consideration. The most significant increases were found among patients with intrahepatic aundice, and among those who had not had biliary obstruction prolonged sufficiently so that the hepatic parenchyma had been greatly injured. In carcinomatous biliary obstruction, the high levels observed may be partially due to the presence of neoplastic masses. In no instance, however, did the value for lactate end of the first hour usually exceed the usual threshold of 50 to 60 mgm. The average increases noted approximated those seen in studies of the experimental animal after hepatectomy, although the patients in question doubtless had an ample reserve of functioning hepatic tissue. In other cases of severe hepatic injury has been found to be associated with normal values for lactic acid.



The compensatory tendency is also noted in work with the hepatectomized animal. Lactose animals are capable of maintaining a fairly normal value for lactic acid for brief periods under resting conditions, but trivial degrees of muscular activity and various extraneous factors which would not affect a normal animal may cause an increase. In other words, the mechanism of lactic acid, although not entirely disrupted, is rendered considerably less stable after removal of the liver. The same is probably true in the more severe degrees of injury to the hepatic cell, under ideal conditions the normal value for lactic acid of the blood is maintained, but it is easily disturbed by any of the large number of factors known to affect it.

The truth of this statement is apparent when one considers the variable findings in clinical cases of hepatic insufficiency. Data concerning the blood lactic acid in such cases, which have been considered in a previous paragraph illustrate how a mechanism rendered unstable by hepatic injury can be affected by extraneous factors which may or may not have to do with hepatic function.

The variations in lactic acid of the blood which follow injection of racemic sodium lactate or lactic acid are worthy of brief mention. In hepatic disease, as in the hepatectomized animal, administration of lactic acid, or of lactate solutions, is apparently followed by utilization of a considerable amount of the injected material. The prompt disappearance of the excess of lactic acid from the blood under these conditions is of interest in view of the suggestion that lactic acid and lactates may be of therapeutic value in some types of hepatic disease. Morawitz has given lactic acid in doses of 10 grams, in cases of hepatic coma due to portal cirrhosis, and on at least one occasion the patient made a rather remarkable temporary recovery. We have had a similar experience with one patient who lapsed into hepatic coma following splenectomy for splenic anemia. Efforts to revive this patient by intravenous injections of solutions of glucose were unavailing. Lactic acid, 10 grams, in 2 per cent solution, was thereupon given daily for three days. The patient promptly recovered consciousness and remained in good condition for a few days, until death occurred as a result of hemorrhage from esophageal varices. In three other cases of marked hepatitis and jaundice, rapid improvement followed daily intravenous administration of this dose of lactic acid or of sodium lactate. All of these patients had been jaundiced for a considerable period of time, and appeared to be losing ground at the time the use of lactic acid was begun. The treatment, of course, is based on purely empirical grounds, but it seems worthy of further study in cases of this type.

#### SUMMARY

Slight to moderate increases in lactic acid of the blood are noted in hepatic disease, particularly significant changes are noted in the more

severe grades of intrahepatic jaundice and in carcinomatous obstruction to the biliary passages. These increases are comparable to those observed after experimental hepatectomy, but in general they do not exceed the renal threshold for lactic acid of 30 to 40 mgm for each 100 cc of blood.

Much greater elevations of values for lactic acid of the blood are found in some cases of clinical hepatic insufficiency, but in these cases factors other than the functional capacity of the liver are responsible for the increases noted. Disappearance of injected sodium lactate from the blood of patients with hepatic disease is delayed in comparison to the rate of disappearance seen in work with normal persons. The results may be correlated roughly with those of bromsulphalein or galactose tests of hepatic function. As compared with the findings in studies upon normal persons, the regulation of blood lactic acid of hepatectomized animals, and of patients with disease of the liver, is considerably less stable, but is not completely disorganized.

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# DIETARY PROTEIN IN HEMORRHAGIC BRIGHT'S DISEASE

## I EFFECTS UPON THE COURSE OF THE DISEASE WITH SPECIAL REFERENCE TO HEMATURIA AND RENAL FUNCTION<sup>1</sup>

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The question of the possibility of injury to the kidneys by the proteins of the diet has received the attention of a number of investigators. Recently Newburgh and coworkers have published some observations which throw new light on the problem. In 1919 Newburgh (14) reported a series of experiments in which renal injury was observed in rabbits to which high protein diets were given. In 1928 a further report (15) was made of experiments in which white rats were given 75 per cent of their diet in the form of proteins, either as liver, or as beef muscle, or as casein. Renal injury was noted in all groups after varying lengths of time. In the group receiving liver, the kidneys at autopsy were enlarged, granular and characterized by both glomerular and tubular lesions and fibrosis. These changes were well marked at the end of "less than a year" of the liver diet. In the case of casein the renal injury was least marked, being confined to the tubules, and required the longest time for its production, while the lesions occurring in the group receiving beef muscle were intermediate between the two extremes both in degree and in the rapidity of their production. In a more recent report Newburgh (16) expressed the belief that the difference in results obtained in previous work was due to the nephrotoxic effects of products of nuclear material and not to the proteins *per se*, since feeding of sodium nucleate produced hematuria. He was able to produce kidney injury with less than 75 per cent of liver in 80 per cent of the animals.

Other workers have reported the production of renal lesions in animals by the feeding of high protein diets. These include Osborne, Mendel, Park and Winternitz (17), and Polvogt, McCollum and Simmonds (18). On the other hand Drummond, Crowden, and Hill (5), Jackson and Riggs (7), and Addis, MacKay and MacKay (3) failed to find renal injury in similar experiments. MacLean, Smith and Urquhart (12) were able to produce renal injury in rabbits by means of high protein diets only when green leaves and vegetables were omitted from the diet.

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<sup>1</sup> Work done under a grant from the Fluid Research Fund of the Rockefeller Foundation

75 and of 150 grams of protein. Urinary sediment counts were made at least twice a week, more frequently when the urine was of high specific gravity and proper acidity. Counts were not done when the urine specimens were not sufficiently acid and of low density. Urea clearance tests were done according to the method of Möller, McIntosh, and Van Slyke (13), between the hours of 7 and 10 A. M., before the patient had risen and while he was still fasting. Urea determinations were made by the gasometric method of Van Slyke (21). The urinary protein was estimated daily by the method of Kingsbury, Clark, Williams and Post (9). The total nitrogen of the urine and stools was determined by the usual Kjeldahl procedure. The stools corresponding to each period were separated by means of carmine. Chemical analyses were done on blood specimens obtained before breakfast according to the following methods: non-protein nitrogen, Folin and Wu (6), serum proteins, Wu and Ling (22).

Patients were weighed daily, before breakfast. Urine and stool specimens were stored in a refrigerator as soon as they were obtained.

#### CASE REPORTS

*Case 1* J. B., number 40617, a boy of 15 years was admitted November 5, 1930. Three weeks before this date a tooth was extracted because of an abscess. Three days later he had a "sore throat" accompanied by mucopurulent nasal discharge. One week before admission he began to have general malaise, headache, vertigo, nausea, vomiting, pain in the lumbar regions, edema of the face and legs, oliguria and hematuria. No previous attack of this nature had been observed. For five or more years he had suffered from repeated attacks of upper respiratory infection characterized by mucopurulent nasal discharge, chronic cough productive of mucopurulent sputum in the morning only and occasional attacks of sore throat. There was no history of scarlet fever.

On examination the temperature, pulse and respirations were found to be normal. The skin was pale, but the mucous membranes were of good color. There was slight, but definite, edema of the face and lower extremities. The eye grounds were normal. The nasal septum was deviated to the left and there was slight, not very definite tenderness over the antra. The tonsils were enlarged with exudate in the crypts. The posterior chain of cervical lymph nodes was palpably enlarged. The examination of the lungs was essentially negative. On percussion the left border of cardiac dullness was found to be 10 cm. from the midsternum. The systolic blood pressure was 170 mm. Hg, diastolic 90 mm. Hg. The liver edge was felt just below the costal margin.

*Laboratory findings* The urine was grossly bloody. It contained a considerable amount of albumin, and many hyaline, granular, and cellular casts. The blood count was as follows: hemoglobin 75 per cent (11.25 grams per 100 cc.), red corpuscles 4,100,000, leucocytes 15,800. Chemical analysis of blood gave the following results: nonprotein nitrogen, 45 mgm. per 100 cc., serum albumin, 4.28 grams per 100 cc., serum globulin, 1.84 grams per 100 cc. The phenolsulfonphthalein excretion was 20 per cent in 2 hours. The Wassermann and Kahn reactions of the blood were negative. A blood culture



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TABLE I  
Case I, J B \*

Date	Period	Diet protein daily	Nitrogen exchange						Blood				Urinary red cells per 12 hours	Urea clearance		Blood pressure	Weight	
			Intake	Output			Balance		Albumin	Serum		Hemo- globin						
				Urine	Feces	Total	Period	Per gram		Globulin								
1880																		
November 5		grams	grams	grams	grams	grams	grams	grams	mgm. per 100 cc.	mgm. per 100 cc.	grams per 100 cc.	grams per 100 cc.	per cent	millions cubic mm.	cc. per minute	cc. per minute	mm. Hg	kgm.
November 12		30-50							45	20	4.8	1.8	75	250	19	47	170/90	64.5
December 4									37	11			70		40		150/70	63.0
December 20																		61.5
1891																		
January 7-13	I	75	83.8	61.2	6.0	67.2	+16.6	+0.2	26	10	5.4	2.5	88	122-225	45	57	130/90	61.2
January 14-20	II	75	83.0	79.0	4.3	77.3	+5.7	+0.0	22	11	5.4	2.5		102-210	47	77	110/85	61.2
January 21-27	III	120	169	147.6	4.6	151.1	+17.8	+1.1	26	9	5.2	2.4		102-204	71	76	110/85	61.2
January 28 to February 3	IV	150	168.5	155.2	4.7	159.9	+8.6	+1.1	40	19	6.1	2.5		125-250	75	70	110/90	62.4
February 4-10	V	150	168.5	155.2	4.9	159.9	+8.9	+1.3	28	16	4.8	2.0		140-250	75		110/90	62.1
February 11-17	VI	150	167.4	153.7	7.8	161.5	+6.7	+1.4	40	18	4.0	2.2		105-135	88	88	110/85	62.7
February 18-24	VII	150	167.6	157.7	4.1	161.8	+1.5	+1.3	43	18	4.7	2.2		80-140	74	79	105/85	63.0
February 25 to March 3	VIII	150	167.8	157.7	4.1	161.8	+1.5	+1.3	39	17	4.6	2.4		110-135	74	86	110/80	63.2
March 4-7	IX	75	48.0	40.5	2.9	52.4	-4.4	-1.1	31	15		2.5	80	60-75	65	88	110/60	64.3
March 8-15		75							34	12	4.3			50-65	51	74		64.4
March 16-31		75							35	10		2.0		15-33	51	71		64.5
April 1-15		75							29	14	4.4			34-61	58	70		65.2
April 16-30		75							28	7				10-39				
May 1-14		75							30	11				12-18				

\* Blood chemistry values, weight and blood pressure are for first day of period. Sediment counts are extreme values obtained during each period. Urea clearance values are the average for all determinations of each period.

$$C_s = \frac{U \sqrt{V}}{B} = \text{"standard" clearance.} \quad C_m = \frac{U V}{B} = \text{"maximum" clearance, i.e. when } V \text{ exceeds 2 cc. per minute}$$

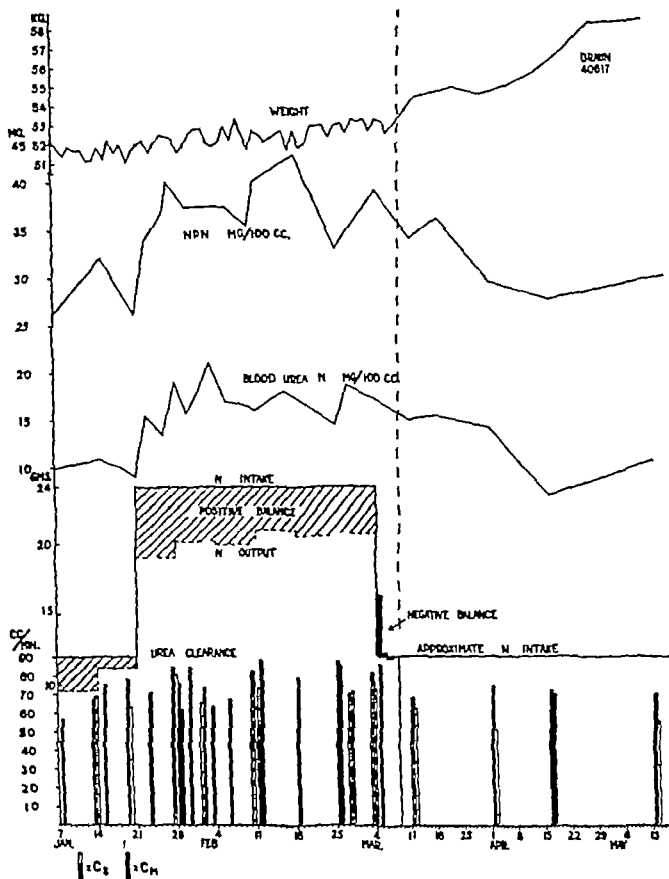


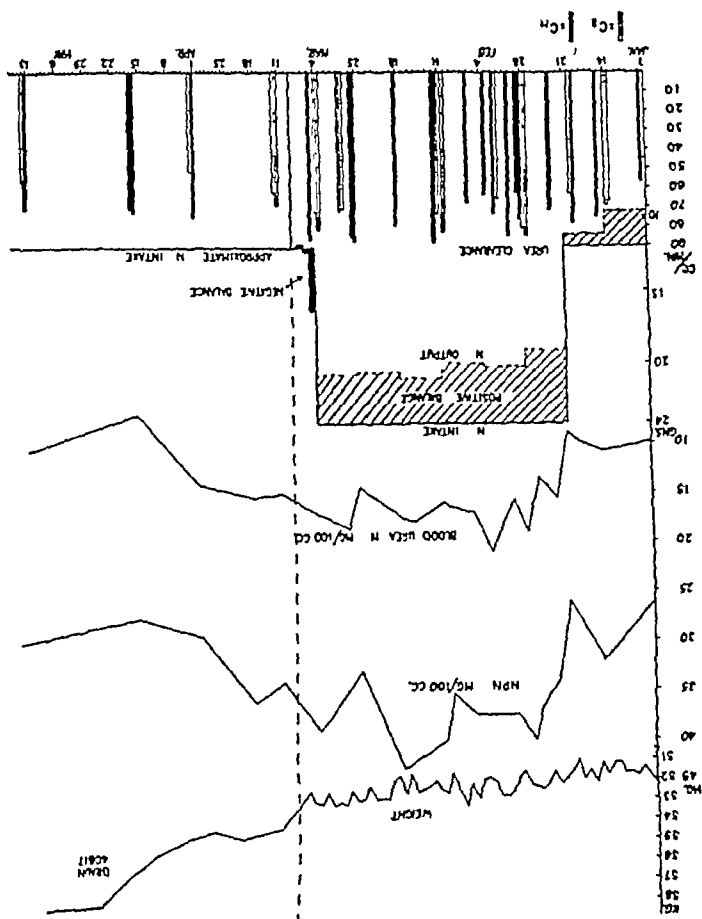
FIG 2 CASE 1 J B DATA COLLATERAL TO FIG 1

$C_s$  is the "standard,"  $C_m$  the "maximum" clearance of blood urea.

The serum proteins fluctuated somewhat without any direct relation to the protein intake. The serum albumin remained within nearly normal limits. The serum globulin was slightly low throughout.

The hemoglobin rose from 70 to 90 per cent during the observation.

The urinary sediment counts revealed a steadily decreasing hematuria in spite of the increase in dietary protein. There was a transient increase in period IV probably due to a slight cold which developed at that time.



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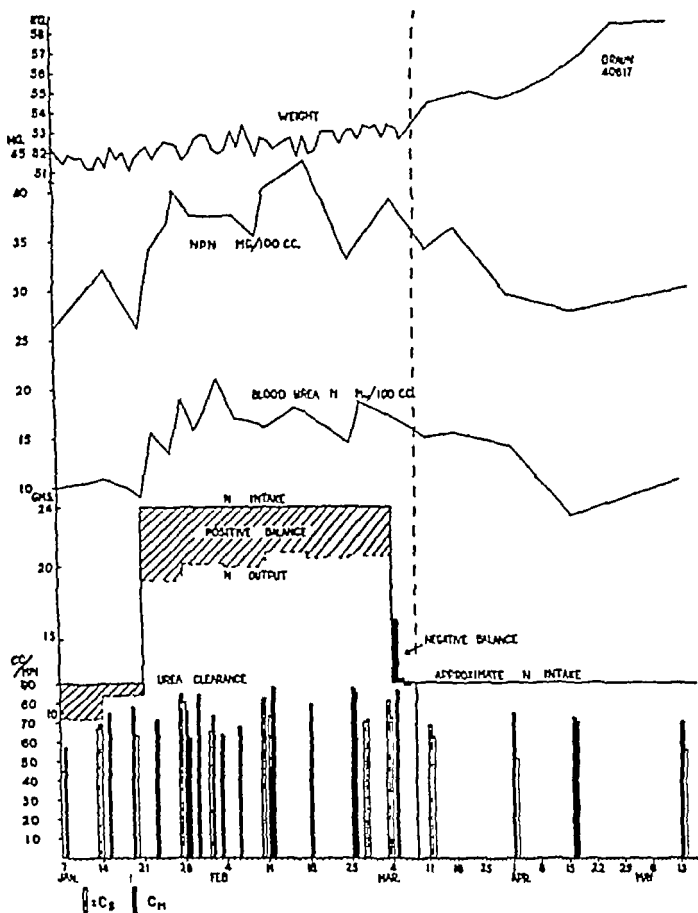


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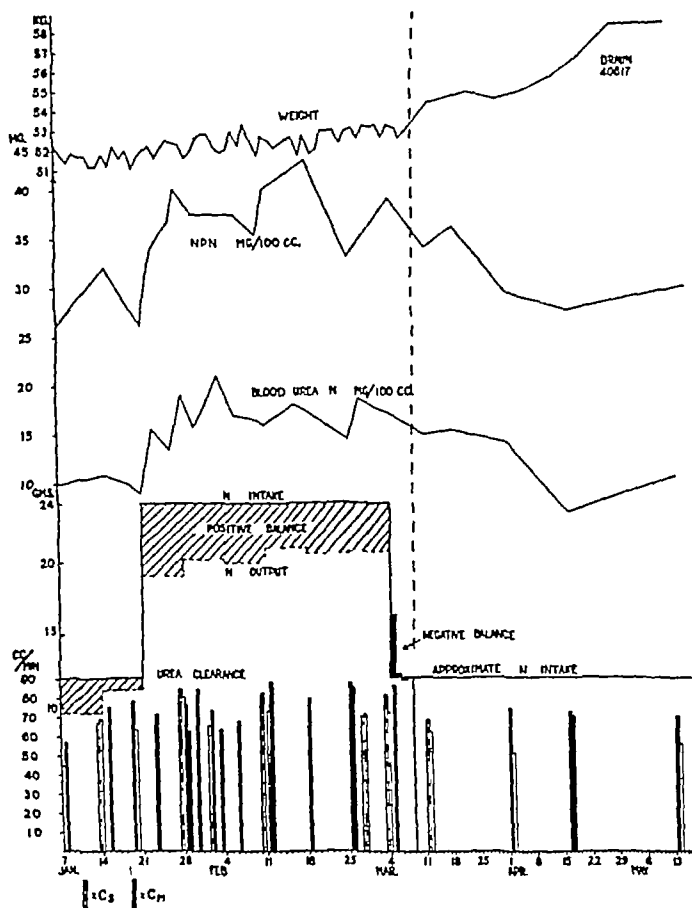


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malaise and fever Ten days before admission there appeared generalized edema, headache, vomiting and epistaxis

On examination temperature, pulse, and respirations were normal There was edema of the face, over the sacrum and lower extremities The cervical lymph nodes were enlarged and tender The eye grounds were normal There was some discharge in the right nostril but no sign of sinusitis was discovered either by x ray, transillumination or puncture of the antrum The ears were normal The tonsils were not large but showed evidence of chronic infection The examination of the heart and lungs revealed no abnormalities The systolic blood pressure was 160 mm Hg, diastolic 100 mm Hg

*Laboratory findings* Hemoglobin 76 per cent (11.4 grams per 100 cc.), red corpuscles 4,630,000, leucocytes 10,400 The Wassermann and Kahn reactions of the blood were negative The urine showed marked albuminuria, and the sediment contained many red corpuscles, hyaline, granular and cellular casts

The nonprotein nitrogen of the blood was 75 mgm per 100 cc

Roentgen examination of the chest revealed slight enlargement of the heart

Six days after admission the patient developed bilateral otalgia and the temperature rose to 38.5° C Large blebs appeared on both drums Bilateral myringotomy was done with the escape of serosanguineous fluid The ears continued to discharge for some time Hematuria persisted, and at times the urine was visibly blood tinged There was a progressive anemia until on December 26, 1930 the hemoglobin was 60 per cent (9.0 grams per 100 cc.), red blood cells 2,800,000, leucocytes 16,000 The ears gradually improved, but hematuria continued, and the blood hemoglobin decreased further to 45 per cent (6.75 grams per 100 cc.), on January 26, 1931 On February 3rd he was given a transfusion On February 12th a tonsillectomy was performed Thereafter clinical improvement was rapid and hematuria decreased

Upon entrance the patient was given a salt poor diet containing 35 to 40 grams of protein daily Edema decreased rapidly during the first few days and thereafter slowly A month later demonstrable edema had entirely disappeared and did not reappear thereafter

On January 29th, fifty days after admission, the protein of the diet was increased to approximately 50 grams Beginning February 28th he was kept on a weighed diet containing varying amounts of protein as indicated in Table 4 On March 8th he was transferred to the metabolism division and remained there until discharge He was in bed until May 5th after which he was allowed up for gradually increasing periods On May 25th he was discharged home on a diet containing about 75 grams of protein We believe that he received approximately this amount daily During this interval he visited the hospital weekly for observations On June 21st he was re-admitted to the metabolism division and immediately given a diet containing 150 grams of protein for a period of 12 days He was discharged on July 2nd and has been under observation in the Out Patient Department since that time.

As in Case 1 the fluid intake had to be quite small on some days in order that Addison's sediment counts might be done The average daily total calculated water intake while the patient was on 40 to 75 grams of protein was from 2700 cc. to 2900 cc. During the first week on 150 grams of protein it was about 3400 cc. daily, thereafter, because of increase in atmospheric temperature it was raised to 4000 cc daily

Laboratory observations on this patient are summarized in Table 3 and Figures 3 and 4





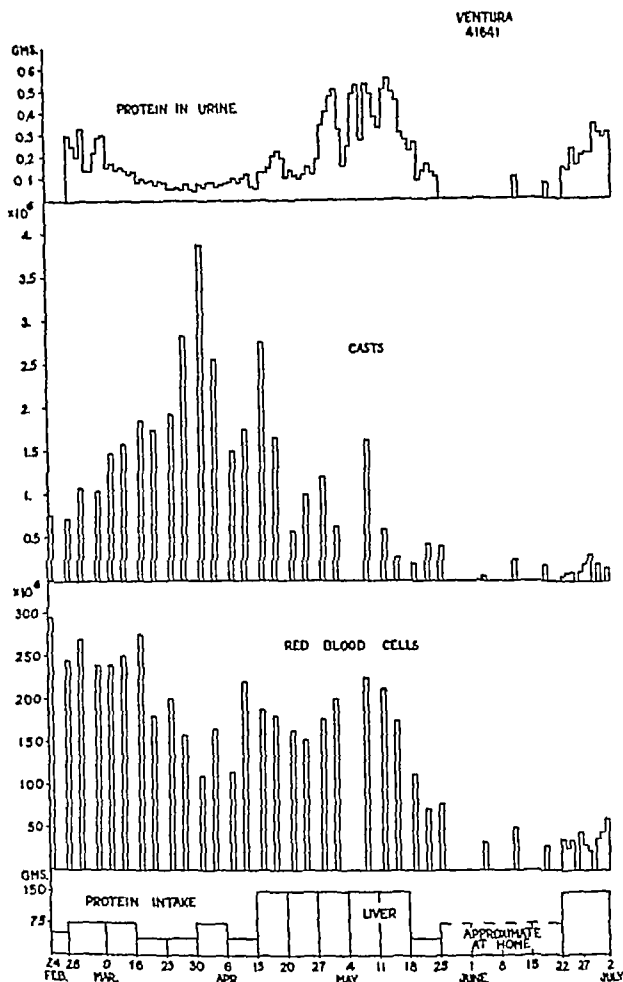


FIG 3 CASE 2 P V CHRONIC HEMORRHAGIC NEPHRITIS

*Comment* The observations made on Case 2 extend over a period of 6 months and 22 days. These are summarized in Table 3. During a fore-period of 77 days the protein of the diet was about 35 to 50 grams. During 12 weekly periods he was observed in the metabolism ward, where the protein intake was varied from 40 to 150 grams per diem.

In period IV the patient came into nitrogen balance during the second week on 40 grams of protein. All periods in which 75 or 150 grams were given showed a positive nitrogen balance. The total caloric intake in all periods varied from 3000 to 3800 calories per diem, the increase being necessary to satisfy the patient's hunger. Carbohydrate and fat were given in nearly equal amounts. The percentage of total calories derived from protein varied from 5 to 18 per cent. Considerable amounts of this protein were derived from beef muscle, but relatively more came from egg and milk than in Case 1. See Table 4.

During periods IX and X beef muscle was replaced by liver.

The total nonprotein nitrogen of the blood rose slightly above normal.

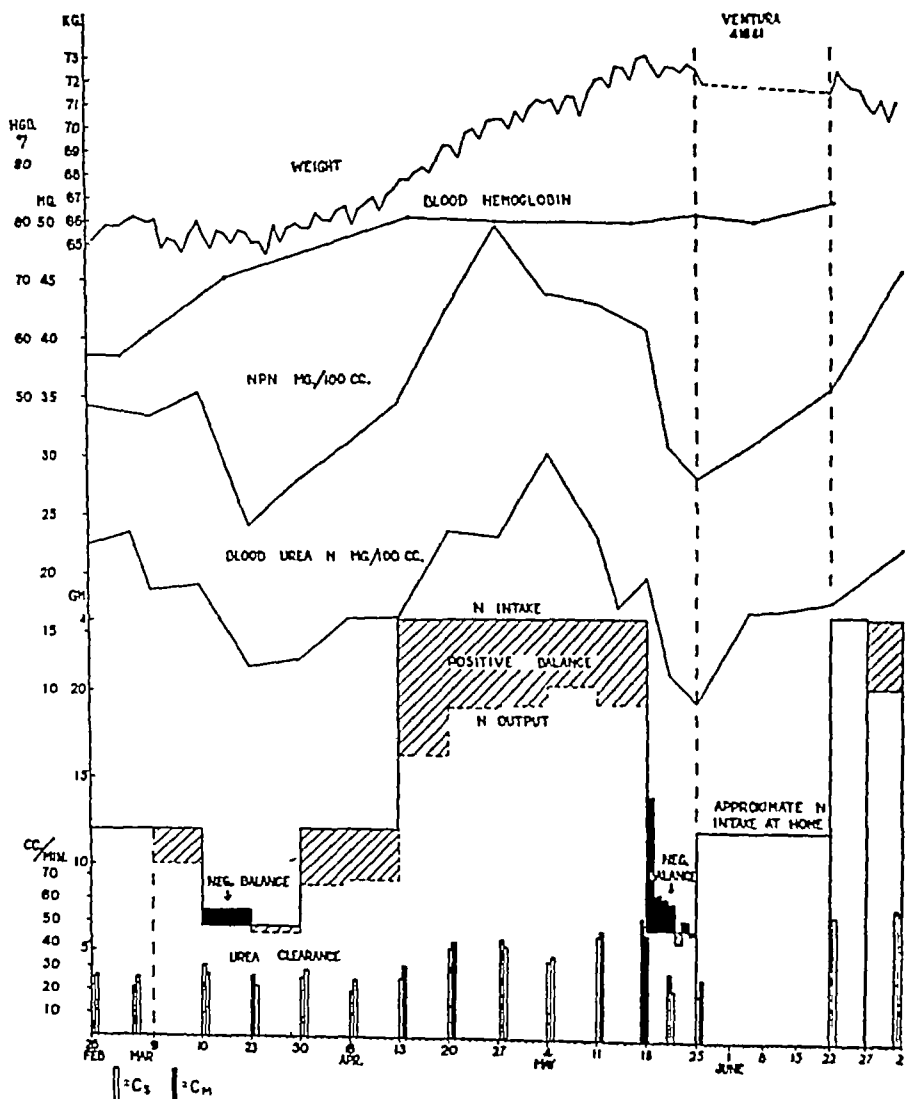


FIG 4 CASE 2 P V DATA COLLATERAL TO THAT OF FIG 3

TABLE 4  
*Analysis of sources of protein in diet of Case 2, P V*

Period	Calories per day	Carbohydrate	Fat	Total protein		Muscle daily	Liver daily	Egg daily	Milk daily	Vegetable daily
				Daily	Per cent of total calories					
		grams	grams	grams		grams	grams	grams	grams	grams
I 9 days	3000			75		25.5		10.4	14.5	24.6
II 7 days	3017	184	210	75	10.2	23.7		18.1	15.6	17.6
III, 7 days	3180	204	234	40	5.1	9.5		10.8	5.7	14.0
IV 7 days	3238	203	241	40	5.1	9.3		10.8	5.9	15.0
V, 7 days	3143	179	226	75	9.8	20.4		18.0	15.7	20.9
VI, 7 days	3213	179	234	75	9.6	20.3		15.6	17.5	21.6
VII 7 days	3850	229	247	150	16.0	60.2		24.1	37.8	27.9
VIII, 7 days	3788	224	242	150	16.2	60.0		25.8	36.7	27.5
IX, 7 days	3457	210	213	150	17.8	10.0	57.5	29.3	26.9	26.3
X, 7 days	3510	211	218	150	17.5	16.6	57.0	27.6	23.2	25.6
XI, 7 days	3743	218	240	150	16.4	60.0		26.0	37.2	26.8
XII, 7 days	3688	204	243	40	4.5	9.4		10.8	6.1	13.7
XIII 5 days	3780	219	243	152	16.5	56.5		28.4	37.2	27.9
XIV, 5 days	3837	230	245	150	16.0	58.5		25.2	39.4	26.9

in periods VIII to XI, but decreased when more fluids were given. The fluctuations in blood urea followed roughly the fluctuations in protein intake.

The serum proteins changed but little. During the fore-period serum albumin was below normal. As the edema cleared it rose to about 4.5 per cent and remained at that level independently of the level of protein ingestion. The globulin remained rather constantly at a slightly low level throughout.

The hemoglobin which had fallen to 57 per cent at the beginning of period I, rose to about 80 per cent during the next four months of liberal protein intake.

The urinary sediment counts revealed a marked decrease in hematuria during the fore-period. From period I on this tendency to decrease was slower but steady and no essential difference was found between periods in which 40 grams of protein were given and those in which 150 grams were taken.

The urinary protein showed some tendency in this case to increase or decrease as the dietary protein increased or decreased.

The urea clearance tests showed a steady tendency to increase. In this case as in the preceding one the standard clearance values were higher in the high protein periods. From beginning to end the urea clearance was more than doubled.

After the fore-period the blood pressure remained entirely within normal limits throughout and showed no tendency to vary in relation to the level of protein intake.

Weight was lost during the fore-period when edema disappeared. During the subsequent periods there was a steady gain in weight due to the deposition of protein and fat.

In summary of Case 2 it may be said that no deleterious effects upon the kidneys were observed during periods of liberal protein intake at levels of 75 to 150 grams per diem. All measurable factors such as hematuria and renal function showed improvement. The slight increases in proteinuria may be disregarded in view of the great improvement in other factors.

*Case 3* R W, number 54426. A 25 year old truck driver was admitted on November 26th, 1931. Previous to the present illness he had always been in good health except for recurrent sore throats. There was no history of scarlet fever or acute rheumatic fever. Two weeks before admission he had a severe sore throat which lasted several days. Four days before admission he developed edema of the face and extremities, oliguria, nausea, vomiting, and weakness. Twelve hours before admission he became stuporous and remained so until being brought to the hospital. Physical examination showed a well developed, semi-comatose man. The temperature was  $37.9^{\circ}\text{C}$ , pulse rate 76, respirations were of the Cheyne-Stokes type. There was edema of the face, extremities, and sacrum. The ocular fundi were thought to show slight edema of the discs but the vessels were considered to be normal. The heart was slightly enlarged to the left. There was a loud systolic murmur at the apex. The blood pressure was 185 systolic and 100 diastolic. The neck was stiff and there was a positive Kernig sign, otherwise neurological examination was negative.

*Laboratory findings* The urine was grossly blood tinged. Blood hemoglobin was 80 per cent (12 grams per 100 cc), red blood cells 4,100,000, leucocytes 20,000. The nonprotein nitrogen of the blood was 60 mgm per 100 cc. The Wassermann reaction was negative in blood and spinal fluid. Cultures of blood and spinal fluid yielded no growth.

*Course in hospital* During the first few days the patient received glucose solution intravenously and subcutaneously. At first the edema remained stationary and then began to decrease slowly. A lumbar puncture on November 29th showed an initial pressure of 420 mm of water. The spinal fluid contained 14,000 erythrocytes per cu mm. On a second lumbar puncture on the following day initial pressure was 300 mm of water. The spinal fluid was xanthochromic and contained 1,000 erythrocytes per cu mm. During the first days of December the stupor cleared gradually, and the blood pressure fell to normal by December 9th. A motor aphasia and weakness of the upper part of face, arm and leg on the right were observed. Tendon reflexes were increased on the right. There were no sensory changes. There was incontinence of urine and feces. During the middle of December he improved slowly. The edema disappeared and did not return. By December 24th the hemiparesis had cleared entirely and there were no peripheral neurological signs. From that time on improvement was rapid. Slight dysarthria persisted until discharge.

Further findings during the period of improvement were as follows. On December 28th roentgenographs of the chest revealed no abnormalities of heart or lungs except that the aortic knuckle was rather prominent considering the age of the patient. Examinations of sinuses and teeth were negative. The phenolsulfonphthalein excretion was 45 per cent in two hours on December 24th.

TABLE 5  
Case 3 Acute hemorrhagic Bright's disease

Date	Diet protein daily	12 hour urinary sediment count		Urine protein daily	Urea clearance		Blood				Blood pressure	Weight	
		Red cells	Casts		C <sub>2</sub>	C <sub>m</sub>	Nonprotein nitrogen	Urea nitrogen	Serum				Hemo-globin
									Albumin	Globulin			
1931 November 26 December 9 26 28	grams	millions	thousands	grams	cc. per minute	cc. per minute	mgms per 100 cc.	mgms per 100 cc.	grams per 100 cc.	grams per 100 cc.	per cent	mm Hg	kgs.
	20-40	+++++	+	+++++			60	33	3.1	1.6	80	185/110	66.2
	30	+++++		+++++			33	17	3.6	2.6		120/70	54.6
		210	185			44					92	120/70	55.6
1932 January 1 4 5 8 11 12 17 19 22 24 26 29 30 February 3 4	30	300	160	2.0		44	27	8	4.1	2.5	90	120/70	57.2
	30			0.9									58.0
	30	310	260	1.1									
	30	410	330	1.5									
	220	120	180	lost	43	53	26	7	4.2	2.5	93	115/80	59.4
	100	260	250	1.6			35	15	4.0	2.2		110/70	61.2
	100			1.8								120/80	62.0
	100	177	110	1.5									62.0
	100	375	280	1.0		55	39	14	4.6	2.3	90		62.8
	100			0.9				18					62.4
	100	184	335	1.1									
	40	290	384	0.4	40	56	25	12	4.6	2.9			
	40	270	250	0.4	46	53		12				120/80	62.8

day gross hematuria appeared and albuminuria increased markedly. Blood hemoglobin was 92 per cent, red blood cells 5,100,000, leucocytes 14,400. Blood nonprotein nitrogen was 30 mgm per 100 cc. The Wassermann reaction was negative. Stereoroentgenographs of sinuses showed no evidence of infection. Roentgenographs of teeth revealed evidence of an abscess at the base of the lower left second bicuspid.

The patient was given a salt poor diet containing 60 grams of protein. Hematuria decreased very slowly at first. On October 21st the bicuspid tooth was extracted. An abscess was found at its apex and *streptococcus viridans*, *streptococcus hemolyticus*, and *micrococcus crassus* were discovered by culture of the apical granuloma.

During the following month (November) hematuria, as indicated by Addis sediment counts, decreased. The protein of the diet was increased to 80 grams and he was discharged on December 12th, 1929. He was in Texas from January until July 1930, at which time he returned to Rochester. He has been followed closely and sediment counts and urea clearance tests have been done periodically. Both of these tests indicate improvement. The routine urine examinations for more than a year past have been negative, but the sediment counts show that red cells are still present in numbers slightly greater than normal.

On December 1, 1929, after he had been on a protein intake of 80 grams daily, he was given a large beefsteak at the noon meal, making his total protein intake for the day 160 grams. Urinary sediment counts on the morning before the large protein meal and the morning after showed no evidence of an increase in hematuria.

Beginning November 9, 1930 he was under observation while on a weighed diet. The protein content of the diet and the laboratory findings for this period are summarized in Figure 6.

During a 10 day period in which the protein intake was increased to 200 grams per diem the number of red corpuscles in the urinary sediment fluctuated within limits which were no greater than during preceding periods in which the protein intake had been maintained at the lower level of 60 to 90 grams per diem.

The urea nitrogen of the blood rose during the periods of higher protein intake. This is partly due to the fact that water ingestion was limited on the days preceding the urinary sediment counts.

The blood urea clearance during the period of high protein remained at the high level which existed in the preceding periods. In the subsequent periods when protein ingestion had been reduced to 60-90 grams per diem, lower values for urea clearance were obtained.

#### SUMMARY

The four subjects of these experiments were all suffering from hemorrhagic Bright's disease. It is believed that this represented a *diffuse* glomerulonephritis in each case, since all exhibited a latent period between the onset of infection and the onset of nephritis, all exhibited impairment of function at some stage, and all had edema.

The dietary experiments were carried out in the early chronic active stages of Cases 1, 2, and 3, and in the latent stage of Case 4.

In cases such as these the two most important objective criteria which

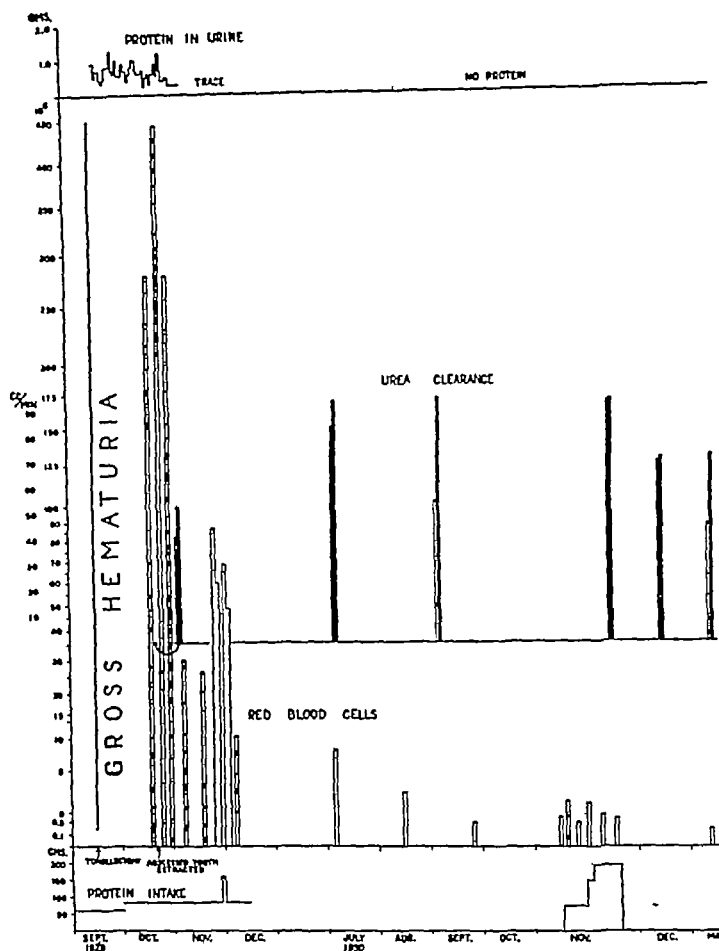


FIG 6 CASE 4 E B CHRONIC HEMORRHAGIC NEPHRITIS IN LATENT STAGE AT TIME OF EXPERIMENT NOVEMBER 1930

may be applied to the observation of the course of the disease are the erythrocyte counts in the urinary sediment and the estimation of renal function by the blood urea clearance tests. The observations of Addis and of Van Slyke have shown that hematuria is the best index of the intensity of the process of disease in the glomeruli, and that the blood urea clearance is the best index of the degree of impairment of renal function.



The possession of a means of evaluating both the intensity and quantity factors in glomerulonephritis makes it possible to study experimentally the factors which modify the course of the disease favorably or adversely

*Hematuria* In the cases which have been presented there is evidence that increasing the protein of the diet from the low level of 40 grams to levels of 75, 150, or even 200 grams, resulted in no significant increases in hematuria. Transient increases in hematuria occurring while the more liberal protein diets were given can be explained in each case by temporary exacerbations of chronic infections. In two of the patients the higher levels were maintained for 5 to 6 weeks at a time, a period long enough to permit deleterious effects to become obvious. It is also clear that the substitution of liver for beef muscle in equivalent amounts for periods as long as two weeks did not increase hematuria.

*Renal function*, as measured by urea clearance, was usually higher during the periods in which more liberal protein intake was given. This may represent the continuance of improvement during the period of the liberal protein ration, and possibly the stimulation of increased functional activity in response to increased demand. In any case there is no evidence of a decrease in functional capacity resulting from a liberal protein allowance.

*Azotemia* was sometimes slightly increased during the periods of higher protein intake. This is due to the fact that water ingestion was maintained at too low a level in order to make possible accurate urinary sediment counts. Toxic symptoms from this cause were never apparent.

The *urinary protein* increased during the higher protein periods in Cases 1 and 2 but not in Cases 3 and 4. The increases were not large, nor were they associated with any other signs of deleterious action.

Visible *edema* was absent during the experimental observations on higher protein diets. It was present in the fore-periods or in previous observations in all cases. The fluctuations in serum proteins were not large, and in no case could they be directly related to the level of protein intake. In Cases 1, 2 and 3 the serum albumin increased as the edema decreased during the fore-periods while moderately low protein diets were being given.

*Blood pressure* was not increased during the higher protein periods as contrasted with the lower. Hypertension was not great in any case, and when it occurred was a transient initial phenomenon of the acute attack.

In every case in which anemia was present improvement in the blood counts occurred.

These experiments are not comparable to those which Newburgh carried out on animals, either in point of duration or as regards the percentage of the total diet consisting of protein. It is not possible to keep human subjects on diets furnishing as great a portion of the energy from protein as Newburgh employed. In our experiments the maximum level was 25 per cent of the total energy of the diet.

The diets with which we have experimented contained more than the usual amount of protein taken spontaneously by the average American. The protein content is greatly in excess of the amount commonly prescribed by physicians in cases of nephritis. It was principally derived from animal sources (Tables 2 and 4).

#### CONCLUSIONS

Four patients with chronic hemorrhagic Bright's disease were observed during periods at different levels of protein intake, ranging from about 40 to 200 grams per diem. No deleterious effects upon the course of the disease were observed during periods of liberal protein intake.

(1) Hematuria, as a measure of intensity of glomerular injury, was not increased.

(2) Functional capacity, as measured by blood urea clearance, continued to increase.

(3) Slight increases in proteinuria which occurred in two cases during the higher protein diets are believed to be without deleterious significance.

(4) Slight increases in azotemia occurred, which were accentuated by the restriction of water intake necessary in making sediment counts.

(5) Serum proteins fluctuated independently of the level of protein intake and of nitrogen balance.

(6) Blood pressure was not increased by the more liberal protein allowance.

(7) General clinical improvement occurred in all cases. Weight increased (not due to edema), anemia improved, and patients returned to their normal state of strength and vitality.

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## STUDIES OF DIABETES MELLITUS

EVIDENCE THAT THE DISABILITY IS CONCERNED SOLELY WITH THE  
METABOLISM OF GLUCOSE THE MODE OF ACTION OF INSULIN

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During the past several years a number of writers in this country and abroad have maintained that liberal additions of carbohydrate to the diabetic diet, usually accompanied by significant reduction in the fat, do not cause glycosuria, nor require an increase in the amount of insulin. They attribute this beneficial outcome to a variety of factors —(1) the depressing effect of fat upon the utilization of glucose, (2) the stimulation of carbohydrate metabolism by ingested carbohydrate, (3) augmentation of the combustion of glucose brought about by under nutrition, (4) and it is even suggested that the improvement is to be attributed to the increased activity of some hitherto unrecognized principle of nutrition brought to light by the high carbohydrate, low fat diet.

It is, for example, stated by Gray and Sansum (1), "That whether the beneficial clinical results which have been obtained by using these diets (carbohydrate-rich, fat-poor) are due to the increase in the carbohydrates or the decrease in the fats, is still debatable." Rabinowitch (2) writes, "Experiences with it (the high carbohydrate, low calory diet) are inconsistent with our present concept of the metabolism of diabetes." Barach (3) maintains that he has "repeatedly seen an increase in dietary fat followed by glycosuria." Later he (4) writes, "There is evidence that insulin applies to the total metabolism, or that one gram of fat creates the need for as much insulin as two grams of carbohydrate." Adlersberg and Porges (5) go so far as to postulate the secretion of insulin as the response of a complex reflex following stimulation of the buccal mucosa by contact with carbohydrate foods.

We believe that this bewildering divergence of opinion and experience has arisen because these investigators have failed to keep two totally unrelated processes apart. They have not taken pains to distinguish sharply between the tolerance of the individual for carbohydrate and the pharmacology of insulin. And yet the former deals solely with a specific attribute of a single person, while the latter is concerned with the behavior of a glandular extract when brought into contact with glucose.

In order to clarify the situation, we have accordingly first centered our attention upon the ability of the diabetic to metabolize carbohydrate

when he receives a high carbohydrate, low fat, low calory diet, and when he receives a low carbohydrate, high fat, high calory diet

Tables 1 to 4, indicate the type of data we have secured Table 1 shows that a middle aged, mild diabetic could dispose of 181 grams available glucose, but not 191 grams when she received a high fat, super-maintenance diet The removal of 100 grams of fat from this diet did not increase her ability to metabolize glucose, since now glycosuria appeared when the available glucose was only 184 grams

TABLE 1  
*Comparison of tolerance on low and on high fat diets*

Hauver Mild diabetes

Date	Protein	Fat	Carbo- hydrate	Calories	Available glucose	Insulin units	Urine sugar
	<i>grams</i>	<i>grams</i>	<i>grams</i>		<i>grams</i>		
January 1	55	190	130	2450	181	0	---
January 2	55	190	140	2490	191	0	---
January 3	55	190	140	2490	191	0	---
January 4	55	190	140	2490	191	0	2 grams
January 7	55	190	140	2490	191	0	2 grams
January 9	55	90	110	1490	151	0	---
January 13	55	90	125	1542	167	0	---
January 14	61	91	140	1618	184	0	---
January 15	61	91	140	1618	184	0	+---
January 16	61	91	140	1618	184	0	2 grams

TABLE 2  
*Comparison of tolerance on low and on high fat diets*

Stoldt Mild diabetes

Date	Protein	Fat	Carbo- hydrate	Calories	Available glucose	Insulin units	Urine sugar
	<i>grams</i>	<i>grams</i>	<i>grams</i>		<i>grams</i>		
February 23	49	51	80	975	113	0	---
February 24	52	51	95	1047	130	0	+--+
February 25	52	51	95	1047	130	0	+++
February 28	30	130	20	1370	50	0	---
February 29	30	130	20	1370	50	0	---
March 10	58	220	50	2484	122	0	---
March 11	58	220	50	2484	122	0	---
March 12	58	220	50	2484	122	0	---
March 13	62	220	73	2520	131	0	---
March 14	62	220	73	2520	131	0	+++

In Table 2, the order of the test is reversed It is shown that a mild diabetic, who received a low fat, low calory diet, had glycosuria when the

available glucose of the diet was 130 grams. A subsequent diet containing more than twice as much fat and only about one fourth as much carbohydrate, quickly abolished the glycosuria. The further striking increase in fat accompanied by only small additions of carbohydrate, was tolerated without glycosuria until the available glucose became 131 grams.

Similar results were obtained with patients whose disease was severe enough to require insulin with any dietary plan. These patients were first placed on a low fat, liberal carbohydrate diet and given much more insulin than needed to prevent glycosuria. The insulin was then slowly decreased without change in diet until glycosuria appeared. The diet was next abruptly changed to the high fat type and the insulin was increased sufficiently to overcome the glycosuria. Whereupon the insulin was slowly decreased again until glycosuria reappeared. A single example will suffice to show our experience with this group. For this purpose we selected a youth who had been under observation by us in the hospital for many months. Table 3 brings out the fact that 16 units of

TABLE 3  
*Comparison of tolerance on low and on high fat diets*

Bryson Severe diabetes

Date	Protein	Fat	Carbo- hydrate	Calories	Available glucose	Insulin units	Urine sugar
	grams	grams	grams		grams		
March 28	47	84	140	1506	174	20-15	---
March 29	47	84	140	1506	174	18-12	---
March 30	47	84	140	1506	174	15-9	---
March 31	47	84	140	1506	174	13-7	---
April 1	47	84	140	1506	174	10-6	---
April 2	47	84	140	1506	174	8-4	+++
April 3	47	84	140	1506	174	10-6	---
April 5	55	220	120	2680	174	10-6	---
April 6	55	220	120	2680	174	8-4	---
April 7	55	220	120	2680	174	8-4	---
April 8	55	220	120	2680	174	6-4	+-
April 9	55	220	120	2680	174	6-4	+++
April 10	55	220	120	2680	174	6-4	7 grams

insulin were sufficient and that 12 units were insufficient to prevent glycosuria, when he was receiving a low fat, low calorie diet, that yielded 174 grams glucose. The abrupt shift to a very high fat, high calorie diet with the same available glucose, required no more insulin to prevent glycosuria. In fact, as the table shows, a slightly smaller dose was now adequate.

Since the experiments just described were of short duration, it might be contended that the prolonged ingestion of the high fat diets would

eventually injure the patient's tolerance. Evidence that this does not take place was published by us (6) as long ago as 1923. Since insulin had not been used in the treatment of the groups of patients studied, it is clear that "downward progress" or loss of tolerance could be dealt with in terms of mortality. We accordingly compared the death rate of our patients who were receiving a high fat, maintenance diet, with the status of other patients treated by competent students who used a low fat, low calory diet. Thus we reported that Williams (7) treated 304 patients with the latter type of diet during a five year period, and had a mortality of 34 per cent, while we gave 176 patients the high fat diet and at the end of four years and four months 25 per cent of them had died. Allen (8) reported the outcome of a three years' trial of the low calory diet in 504 patients, the mortality was 17.1 per cent. During the same period we gave the high fat diet to 137 patients with a mortality of 18.8 per cent. Joslin (9) published his statistics for 536 patients who had taken the low fat, low calory diet from April 1, 1919 to December 31, 1922. The mortality was 23 per cent. During the same interval of time we treated 124 patients by means of the high fat, maintenance diet, with a mortality of 21 per cent. Only one conclusion could be reached.

Nevertheless, the question has recently been reopened, and it is accordingly worth while to cite further evidence that the persistent use of the high fat diet does not reduce the ability of the patient to metabolize glucose.

Dr. F. J., aged 35, first came under our care for the management of diabetes mellitus in 1920. He has taken a high fat diet continuously to date. From 1927 to the present, the diet has consisted of 73 grams of protein, 272 grams of fat, 68 grams of carbohydrate. Twenty-six units of insulin prevented glycosuria for four years. However, early in 1931, an acute upper respiratory infection made it necessary to increase the insulin temporarily to 50 units. Subsequently it was slowly decreased until he was again using 26 units daily without glycosuria.

V. B., aged 19, tolerated a diet consisting of 40 grams of protein, 240 grams of fat, and 30 grams of carbohydrate during a two weeks' trial in the hospital, in May 1931. He continued to take this very high fat diet with a fatty acid, glucose ratio of 3.2 and an available glucose of 77 grams, for six months, when he returned at our request. In the interval he had gained 10 kilograms in weight. In spite of these conditions his tolerance was unchanged.

We have cited the first example to show that the continued ingestion of a high fat diet for many years does not injure tolerance. The record of the second patient is evidence that a diet not only strikingly high in fat, but also one that permitted rapid gain in weight, was likewise without effect on tolerance.

These present studies have merely confirmed our earlier experience with many patients, that the capacity of a diabetic individual to dispose of

the available glucose of a diet, without glycosuria, is unrelated to either the fat or the energy content of the diet. The tolerance of a diabetic is the maximum number of grams of glucose from all sources that can be oxidized in twenty four hours without insulin, and after he has had full opportunity to recover from interfering factors. It has been shown, over and over again, that tolerance is independent of the character of the diet.

We next took up the second question, that is, whether the ability of injected insulin to metabolize glucose is influenced by the composition of the diet. As a basis for this work, we had the enlightening studies of Campbell and of Allan. The substance of Allan's (10) investigations is contained in Table 4. Examination of the first section of the table shows

TABLE 4  
*Depancreatized dog*  
(From F N Allan *Am J Physiol*, 1924, *lxvii*, 287)

Insulin units	Glucose		Ratio glucose : insulin	Glucose equivalent
	Available	Metabolized		
	<i>grams</i>	<i>grams</i>		
40	131	124	3.3	3.1
32	131	112	4.1	3.5
24	131	96	5.5	4.0
20	131	116	6.6	5.8
20	82	74	4.0	3.7
20	132	116	6.5	5.8
20	182	150	9.0	7.5
32	82	80	2.6	2.5
32	132	122	4.1	3.8
32	182	150	5.7	4.7

that the depancreatized dog, who daily received a diet that yielded 131 grams of glucose, metabolized relatively more of it as the insulin was reduced from 40 to 20 units. In the fourth column the relation between the available glucose and the insulin, is expressed as a ratio, while the last column shows how much glucose was disposed of by each unit of insulin. It will be seen that as the glucose increased in proportion to the insulin, so did the amount of glucose that was metabolized per unit of insulin. In sections two and three of the table, the procedure is reversed. Nevertheless, when the relation between dietary glucose and insulin is expressed as a ratio, it is again clear that an increasing ratio is attended by an increasing efficiency of insulin.

Entirely analogous evidence may be obtained from human diabetics. As indicated in Table 5, a well controlled young diabetic could tolerate 76 grams of available glucose without insulin. With each subsequent



TABLE 5  
*Glucose metabolized per unit of insulin with increasing glucose intake*

Bryson

Total	Glucose beyond tolerance	Insulin	
		Units	Efficiency
<i>grams</i>	<i>grams</i>		
76	0	0	—
90	14	10	1 4
106	30	10	3 0
123	47	10	4 7
137	59	12	5 0
174	98	14	7 0
274	198	30	6 8

increase in the available glucose, insulin was also increased enough to surely prevent glycosuria. This amount was then slowly decreased until glycosuria appeared. Column 3 shows the least amount of insulin that would prevent glycosuria for each level of available glucose. It will be seen that as the glucose increased beyond tolerance, each unit of insulin disposed of a greater amount of glucose, until a maximum was reached beyond which further additions of glucose were without effect on the efficiency of insulin.

Experiments with the depancreatized dog and with the human diabetic agree in showing that the glucose equivalent of a unit of insulin is not a fixed quantity, but that it is dependent upon the absolute amount of glucose to be acted upon. The glucose equivalent may be strikingly augmented by increasing the available glucose in proportion to the insulin. This gives a high ratio. However, the reduction of the insulin in the presence of a small amount of glucose, which also increases the ratio, does not increase the efficiency. Hence, there must always be a large amount of glucose present, in order to obtain a high efficiency of insulin. The data also makes it clear that there is a definite upper limit to the amount of glucose that can be disposed of by a unit of insulin. The evidence at hand suggests that this maximum is about 7 grams of glucose per unit of insulin.

These investigations afford a quantitative basis for comparing the required dose of insulin when different types of diet are employed. For example, it is found that a hypothetical patient whose tolerance is 100 grams of available glucose, requires 14 units of insulin when he receives a diet containing 60 grams of protein, 190 grams of fat and 66 grams of carbohydrate. Since the total glucose of this diet is 120 grams, he will be receiving 20 grams more than his tolerance. Accordingly, 14 units of insulin disposes of 20 grams of glucose. The glucose equivalent is therefore 1 4 gram. The diet is now changed to 60 grams protein, 40 grams of

fat, and 150 grams of carbohydrate. The total glucose of this diet is 189 grams, which is 89 grams beyond tolerance. This additional glucose may also be completely utilized without increasing the insulin, since the required efficiency of 6.4 grams per unit of insulin has been demonstrated to occur. But the second diet yields only 1356 calories. If the first diet, that contains 2214 calories, is maintenance, the patient will obtain the extra calories when he takes the second diet by oxidation of about 95 grams of his body fat. The 9 extra grams of available glucose will not cause glycosuria if the efficiency of insulin may be relied upon for 7 grams of glucose per unit.

The study also shows that tolerance is independent of the type of diet. Accordingly, this patient's tolerance will not be increased by adding carbohydrate to the diet, nor diminished by increasing the fat. Such being the case, it is not necessary to use insulin at all in the treatment of this patient, since his tolerance of 100 grams of glucose permits him to obtain a satisfactory diet that will yield the desired calories. A diet consisting of 40 grams of protein, 208 grams of fat and 45 grams of glucose, will contain 89 grams of available glucose and 2212 calories.

#### SUMMARY

1 The tolerance of a diabetic individual is defined as the maximal capacity to dispose of the available glucose from all sources, without glycosuria, in the absence of insulin. This value is not depressed by dietary fat nor augmented by dietary carbohydrate. The continued administration of high fat, maintenance diets does not lower it.

2 The efficiency of insulin in the case of the human diabetic, as in the depancreatized dog, is related to the total amount of glucose upon which it acts. When the available glucose far exceeds the tolerance, each unit of insulin will cause the oxidation of six or seven grams of glucose. Under otherwise similar conditions except for a small excess of available glucose, only one or two grams of glucose are oxidized per unit of insulin.

3 A sharp maximal efficiency is also revealed.

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# A COMPARATIVE STUDY OF THE EXCRETION OF WATER AND SOLIDS BY NORMAL AND ABNORMAL KIDNEYS

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(Received for publication May 25, 1932)

A previous report by us (1) presented evidence to show that normal kidneys are able to excrete concentrated urine, while diseased kidneys are not. The manner in which this information was obtained was as follows. The subjects were on a constant diet for a preparation period of 3 days. Fluid intake was restricted to 1500 cc. daily. Beginning at 6:00 P M of the third day, all intake of food and fluid was stopped for 18 hours. Urine was collected at intervals during this fast and the specific gravity determined. It was found that normal kidneys were able to concentrate the urine to a specific gravity of 1.026 or above. Diseased kidneys were unable to reach 1.026.

In order to observe these facts in more detail, further studies (2) have been made. This time a greater load was imposed upon the kidneys, but for a shorter period of time. The procedure was as follows. Beginning at 10:00 P M, all intake of fluid and food, except a special diet, was withheld for 38 hours. Urine was collected at intervals during this period and the specific gravity determined.

In the previous study, the total available water was approximately 2800 cc. daily. In the latter one, the total available water was reduced to 700 cc. daily.

The solid intake of the body was practically the same in both studies.

Under these latter conditions, it was found that normal kidneys are able to concentrate the urine to a specific gravity of 1.029 or above. Diseased kidneys cannot reach 1.029. The more severe the renal damage is, the lower the concentrating ability of the kidneys was found to be.

The specific gravity of any solution is an expression of a ratio between water and solids in solution. As the kidneys become unable to concentrate the urine, the ratio of grams of water per gram of solid excreted must increase. Figure 1 illustrates this ratio of water to total solids in the urine at different specific gravities. This ratio holds for each specific gravity regardless of whether it is the maximal specific gravity attainable or that of a specimen obtained under submaximal conditions.

Since, in disease, there is an increase in the proportion of water per gram of solid excreted, it is important to determine what effect this high ratio has on the total 24 hour excretion of water and solids.

The lower specific gravity in disease could be brought about in several ways. First, the total volume of urine excreted per unit of time might be normal, but the amount of solids contained therein be below the normal. In this case, there necessarily would be retention of excretory wastes. Second, the amount of solids excreted per unit of time might be normal but contained in an unusually large volume of urine. Under these circumstances, no retention of solids could exist. Third, there might be a combination of both the above possibilities, a decreased excretion of total solids in an increased volume of urine per unit of time.

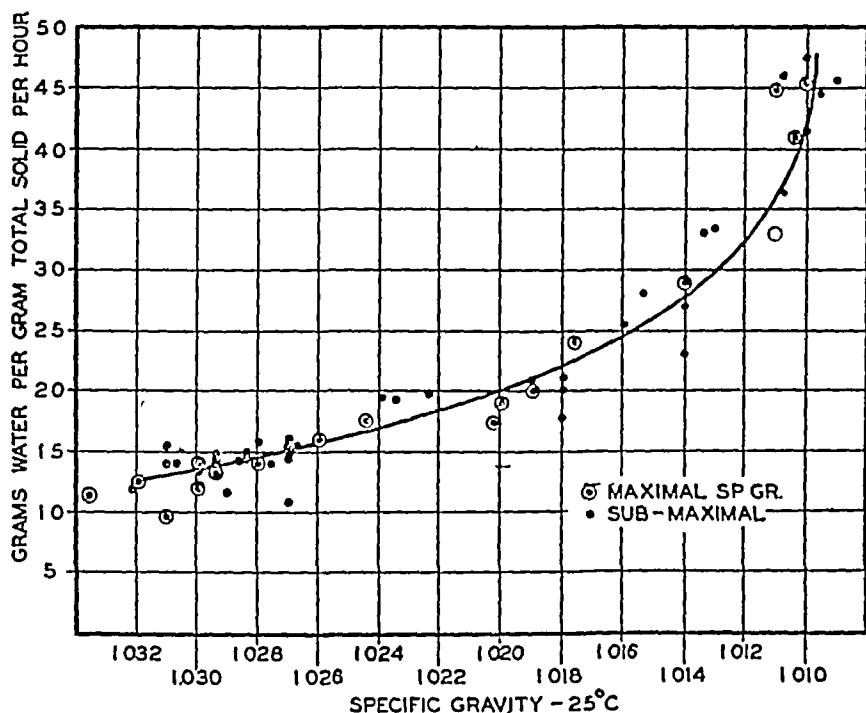


FIG 1 RELATIONSHIP OF WATER TO TOTAL SOLIDS IN THE URINE AT DIFFERENT SPECIFIC GRAVITIES

These studies were done in order to determine what the conditions are in the case of diseased kidneys characterized by a low concentrating ability.

Both normal individuals and those with various types of renal disease were studied. The latter included cases of acute nephritis, chronic nephritis with edema, chronic nephritis with hypertension and no edema, essential hypertension with mild renal involvement, pyelonephritis, and tuberculosis of the kidneys.

The intake of solids was identical in every case. This consisted of a special diet containing protein 40 grams, fat 104 grams, carbohydrate 204 grams, and 1900 Calories. One gram of sodium chloride was added.

Such a diet contains 6 536 grams of nitrogen, 9 1 grams of inorganic solids and 3 286 grams of chlorine The menu of this diet is as follows

<i>Food</i>	<i>Grams</i>	<i>Approximate Measure</i>
Breakfast		
Corn flakes	15	$\frac{1}{2}$ cup
Bread (toast)	60	2 slices— $\frac{1}{8}$ inch
Butter	20	2 squares, or 1 level tablespoon
Cream, 40 per cent	50	$\frac{1}{4}$ cup
Sugar	13	1 tablespoon
Lunch		
Beef steak	100	$4 \times 4 \times \frac{1}{2}$ inch
Potato (baked)	80	1-2 $\frac{1}{2}$ inch diameter
Crackers	16	4
Butter	20	2 squares, or 1 level tablespoon
Dates	70	10
Dinner		
Potato (baked)	80	1 as above
Lettuce	100	$\frac{1}{4}$ head
Crackers	16	4
Butter	20	2 squares, or 1 level tablespoon
Dates	30	4
Peaches (canned) (no juice)	85	1 half
	<hr/> 755	

The total water available when such a diet is fed is approximately 700 grams per 24 hours This was calculated as follows The water of the food was determined by desiccation The average for 16 menus was 372 grams The water of oxidation of the diet alone is 250 grams However, this diet is submaintenance for many individuals, consequently body protein and fat must be oxidized Thus the actual water of oxidation would be greater than that coming from the diet alone. A total water and energy exchange was obtained from 3 normal and 6 abnormal individuals in the series by the method described by Wiley and Newburgh (3) It was found that the amount of water available from all sources when such a diet is fed averaged 706 grams per 24 hours for this group

Beginning at 10:00 P M the night before each experiment, all intake of fluid and food, except the special diet, was withheld for 34 hours From 8:00 A M the following morning until 8:00 A M 24 hours later, all urine was collected as one specimen During this 24 hour period, the special diet was taken

Total solids and water of the urine were determined by the usual method of freezing and then desiccating over sulphuric acid Specific gravity was determined at 25° C by the weight method, using weighing bottles of about 5 cc capacity

When the above conditions were imposed, two distinctly different responses were observed (Table I) These responses divided the subjects into two groups The critical feature was the presence of that well-

TABLE I

*Effect of water restriction on the excretion of water and total solids by normal and diseased kidneys*

Normal specific gravity	Case number	Urine water	Urine solids
		<i>grams per 24 hours</i>	<i>grams per 24 hours</i>
1 032 to 1 029	1	288	25 00
	2	354	25 26
	3	384	33 34
	4	432	
	5	513	37 23
	6	550	33 30
	7	554	39 32
	8	574	37 56
	<i>Average</i>	<i>456</i>	<i>32 95</i>
Abnormal—Group A			
1 028 (incl ) to 1 025	9	515	29 87*
	10	550	32 15*
	11	638	37 64*
	12	675	39 82*
	<i>Average</i>	<i>594</i>	<i>34 94</i>
1 024 (incl ) to 1 020	13	530	25 41*
	14	650	35 75*
	15	655	32 91
	17	672	32 25*
	18	815	41 56*
	19	825	46 20*
	20	847	44 43
	<i>Average</i>	<i>713</i>	<i>41 22</i>
1 019 (incl ) to 1 015	21	600	25 80*
	22	770	31 57*
	23	770	36 98
	24	1224	51 50*
	25	1495	53 23
	<i>Average</i>	<i>971</i>	<i>39 81</i>
1 014 (incl ) to 1 010	27	1000	29 00*
	28	1176	25 67
	29	1440	43 08
	30	1804	33 00
	<i>Average</i>	<i>1355</i>	<i>32 68</i>
Abnormal—Group B			
1 0203	26	247	13 82
1 0096	31	845	18 89

\* Calculated from specific gravity (?)

known type of generalized edema that so characteristically appears as the first important sign of chronic, progressive kidney disease in young adults. The two groups are as follows:

A. Those subjects having renal damage without the nephrotic type of edema. (1) Chronic nephritics who never had edema, or in whom it had disappeared. Cases 9, 10, 11, 13, 15, 18, 20, 22, 23, 28, 29 and 30. (2) Essential hypertension with mild renal involvement. Cases 12 and 14. (3) Pyelonephritis. Cases 17, 19, 21 and 27. (4) Renal tuberculosis. Case 24. (5) Severe secondary anemia with mild renal damage and edema. Case 25.

B. Those subjects with chronic nephritis and the nephrotic type of edema. Cases 26, 31.

In Group A, the most striking deviation from the normal was the larger output of water. With each lowering of the specific gravity, a greater water excretion per gram of solid occurred. Inasmuch as a normal amount of solids was almost always excreted, the increased water output apparently compensated for the decreased ability of the kidneys to concentrate.

Since this group ingested the same amount of water as the normal group, but excreted more water, the extra urinary water must have been released from the body itself.

The subjects in Group B had edema of the nephrotic type. The concentrating ability of both of these subjects was below normal as was the case in Group A. In these patients, however, the response was different. The amount of water excreted was distinctly lower than that of Group A with corresponding specific gravities. In fact, the water excretion of Case 26 was even lower than that of the normal group. With the lowered water excretion, there was also a very low solid excretion.

In Group A, Cases 20 and 22 had the same type of nephritis as Cases 26 and 31 of Group B. However, the nephrotic edema which had been present in the former cases had disappeared before the experiment. Cases 20 and 22 responded to water deprivation by excreting a large amount of water, while Cases 26 and 31 did not. Other subjects in Group A (Cases 25 and 28) with edema which was not of the nephrotic type also excreted a large amount of water.

One might conclude that when nephrotic edema is present, the patients are less able to excrete water and solids. This would account for the presence of the edema and the lowered excretion of water and solids. But, as will be shown later, both Cases 26 and 31 could excrete water and solids. Hence, when nephrotic edema is present, the body water is more firmly held than in any of the other types studied, normal, "dry" nephritics of any type, cardiac edema and edema associated with severe anemia.

In order to compare the effects of "water restriction" with the administration of extra water, another study was made (Table II). Three nor-



TABLE II

*Comparison of the effect of restriction and administration of water on the excretion of water and total solids by normal and diseased kidneys*

Case number	Diagnosis	Part A, water restricted		Part B, water as desired	
		Urine water	Urine solids	Urine water	Urine solids
		grams per hour	grams per hour	grams per hour	grams per hour
5	Normal	17 90	1 266	21 36	1 551
16	Normal	20 74	1 505	27 79	1 428
8	Normal	23 93	1 565	60 50	1 655
	<i>Average</i>	20 86	1 445		1 545
30	Chronic nephritis with hypertension, no edema	75 16	1 375	205 10	1 572
28	Chronic nephritis with hypertension, cardiac failure, edema present	48 99	1 069	88 00	1 148
31	Chronic parenchymatous nephritis, edema moderate	35 20	0 787	67 62	1 249
26	Chronic parenchymatous nephritis, edema marked	8 78	0 505	164 50	1 445
25	Anemia—secondary, severe—edema moderate	55 07	1 676	62 28	2 218

mal and 6 abnormal subjects of the above series were studied. The latter were selected because of the various types of renal disease which they represented.

The same conditions were maintained as previously described except that as shown in part B (Table II), extra water was allowed as desired.

When the normal group were allowed extra water, the average solid excretion was 1 545 gram per hour. This was not strikingly greater than when the available water was small.

When the abnormal group were allowed extra water there was a striking increase in the water excreted. With the increased water excretion, there was also a marked increase in the solid excretion. This response was the same in the various types of disease, whether edema was present or absent and whether the edema was of the nephrotic type or otherwise. It should be especially noted that Cases 26 and 31 increased the water excretion remarkably and with it a solid excretion approaching the normal.

Case 26 presents striking evidence in support of the view that retention of solids is to be attributed solely to an insufficient intake of water. Since the low concentrating ability necessitates an increased volume of urine to remove the urinary wastes, and since body water is not easily given up in nephrotic edema, the only chance of preventing retention of urinary solids is by the ingestion of a really large amount of water.

## SUMMARY

Individuals with renal damage are unable to form as concentrated urine as normal. This necessarily means that the urine in the former has a high water/total solid ratio. It naturally follows that if the individual is to excrete the normal amount of solid waste products per 24 hours, he can accomplish this only by means of a large volume of urine. The lower the concentrating ability of the kidneys, the greater must the volume of urine be if no retention is to occur.

When water is restricted there are two distinctly different responses by individuals with renal disease. In the first group are those individuals having renal disease unaccompanied by the nephrotic type of edema. This group has a large volume of urine per 24 hours in spite of the small intake of water. This extra urine water is released from the body itself. In the second group are those individuals having renal disease accompanied by the nephrotic type of edema. This group has a small volume of urine per 24 hours. This is not due to the inability of the kidneys to excrete water. It is due to the unusually great affinity of the tissues for water.

When water is freely allowed, there is no difference in the response of the two types. Increased water ingestion is followed by both an increased water excretion and solid elimination. This response is the same whether edema is present or absent and whether this edema is of the nephrotic type or otherwise.

Apparently the process of excretion of waste products in renal disease is fundamentally one which avoids the retention of solids by increasing the water output as a compensation for a low concentrating ability. If the amount of ingested water is too small for this purpose, body water may be released. If the body water is not released, then the process fails and retention of wastes results. In either case, an extra large amount of ingested water is needed to insure the complete removal of solid wastes.

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## STUDIES ON THE PHYSIOLOGY OF THE PARATHYROID GLANDS

### V ACTION OF PARATHYROID EXTRACT ON THE RENAL THRESHOLD FOR PHOSPHORUS

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The most striking end result of the administration of the parathyroid hormone, and the one most important therapeutically, is the elevation of the serum calcium. The present paper is the fifth of a series (1) (2) (3) which have been largely concerned with the mechanism by which that result is achieved.

As previously emphasized, the four cardinal metabolic abnormalities which a lack of parathyroid hormone occasions are 1 Fall of urinary phosphorus excretion 2 Rise of serum inorganic phosphorus 3 Fall of serum calcium 4 Fall of urinary calcium excretion.

Similarly, the giving of parathyroid extract corrects these abnormalities, causing 1 Rise of urinary phosphorus excretion 2 Fall of serum inorganic phosphorus 3 Rise of serum calcium 4 Rise of urinary calcium excretion.

Many chemical investigations dealing with calcium and phosphorus metabolism have shown that the two substances are closely interrelated. It, therefore, seems probable that the parathyroid hormone effects one of the four changes mentioned above and that the other changes are sequelae. In previous experiments it appeared that after parathyroid extract administration the alteration in urinary calcium was very much delayed (1), whereas the increase in phosphorus excretion was immediate (1). This suggested the hypothesis that the phosphorus changes produced by the parathyroid hormone were primary and that the calcium changes followed them. In the earlier experiments, however, the analyses of blood serum, after injections of parathyroid extract, showed, as a rule, that by the time the serum phosphorus had fallen the serum calcium had already begun to rise. In the present experiments, which confirm the previous observations, the changes in blood and urine were examined more closely, with the result that some additional observations have been made.

## EXPERIMENTS

Four nearly identical 8-hour experiments were performed on two patients suffering from postoperative hypoparathyroidism. Both were on the metabolism ward of the Osler Clinic. They were fasted and kept at rest for twelve hours before and during the whole time of the experiment. One hundred cc of water was given each hour during the experiment. Hourly urine specimens were collected for three hours before and five hours after the injection of the parathormone<sup>1</sup>. This was given intramuscularly and the site massaged for five minutes. Blood specimens were taken just before the extract was given and one-half, one, two, and either four or five hours afterwards. Precautions were taken to avoid venous stasis, the serum was separated after allowing one-half hour for clotting, the phosphorus filtrate was made at once. Serum calcium was estimated by the method of Kramer and Tisdall (4), inorganic phosphorus by that of Fiske and Subbarow (5).

The ultrafiltration experiments were carried on upon large samples of blood taken while fasting. Dry negative cotton in alcohol-ether was used as the substance for membranes. The technique and precautions described by Grollman (6) were followed.

It is perhaps useful to emphasize the method by which the filtrate was obtained for analysis. Ten cc of serum was suspended in a collodion sac and enclosed in a glass chamber, which contained only a single small hole at the top. A pressure of 180 mm Hg was applied. As soon as the membrane became damp on the outside, the moisture was removed by wiping with an ash-free filter-paper. As filtration proceeded, each 0.3 to 0.4 cc coming through was separated and analyzed. A constant concentration was obtained usually in the third and fourth samples, samples one and two being slightly higher or lower in phosphate content. The constant value was thus obtained when less than one cubic centimeter had filtered. This minimizes one objection to ultrafiltration, namely the possible alteration in the filtrability of one ion by gross change in concentration of other ions.

## OBSERVATIONS

In all experiments the parathormone injection was followed by a marked rise in urinary phosphorus excretion (Charts 1, 2). This was conspicuous in the first-hour specimen and even more marked in the subsequent specimens. There was a pronounced rise in the phosphorus *concentration* as well as in total amount.

There was a fall in serum inorganic phosphorus, apparent as early as one-half hour after the parathormone injection.

There was a rise in serum calcium, but, whereas the fall of serum inorganic phosphorus was immediate, the rise in serum calcium was delayed. In Experiments 1 and 2, the delay was slight, but in Experiments 3 and

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<sup>1</sup> Parathyroid extract isolated by Collip. Prepared by Eli Lilly and Co.

4 there was no rise in serum calcium during five and four hours respectively after the parathormone injection. At the end of twenty-four hours after the injection the serum calcium had risen as usual.

Serum, subjected to ultrafiltration, showed that 97 to 100 per cent of the inorganic phosphorus was filtrable before parathormone was given (Table 2).

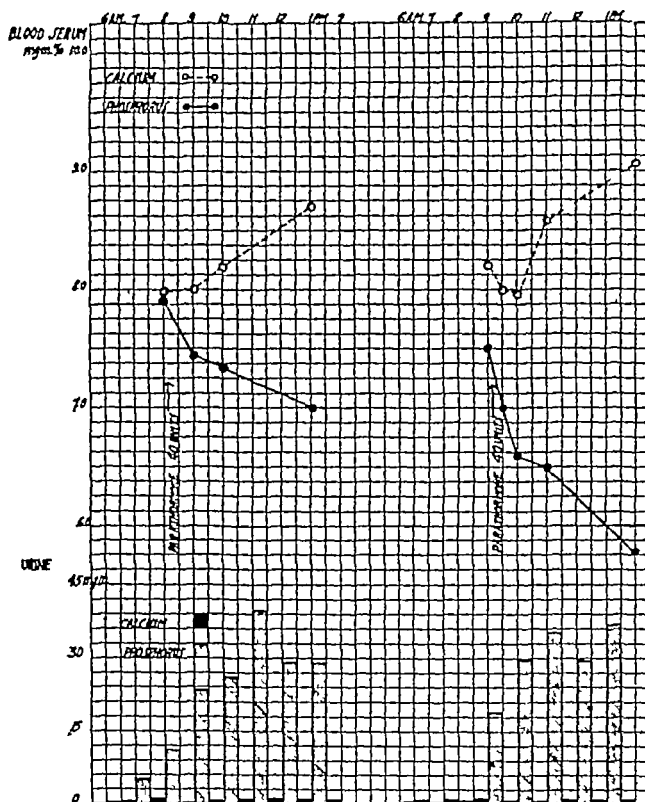


CHART 1 SERUM AND URINARY CALCIUM AND PHOSPHATE IN SUBJECT A C

#### DISCUSSION

In the present experiments, as in those previously reported, the injection of parathormone was followed by a rapid excretion of phosphorus in the urine. This we have observed repeatedly. Although there was sometimes a slight increase in fluid output (Experiments 2 and 4), there

was an enormous increase in phosphorus concentration in the urine, as well as in the total hourly phosphorus excretion

The phosphorus diuresis was accompanied by a fall in serum inorganic phosphorus. It seemed desirable to obtain a rough figure for the total amount of phosphorus lost from the plasma in order to compare it with the amount of phosphorus found in the urine. These figures were obtained as follows. From the data of Chang and Harrop (7) for blood volumes, normal individuals with the surface areas of our subjects

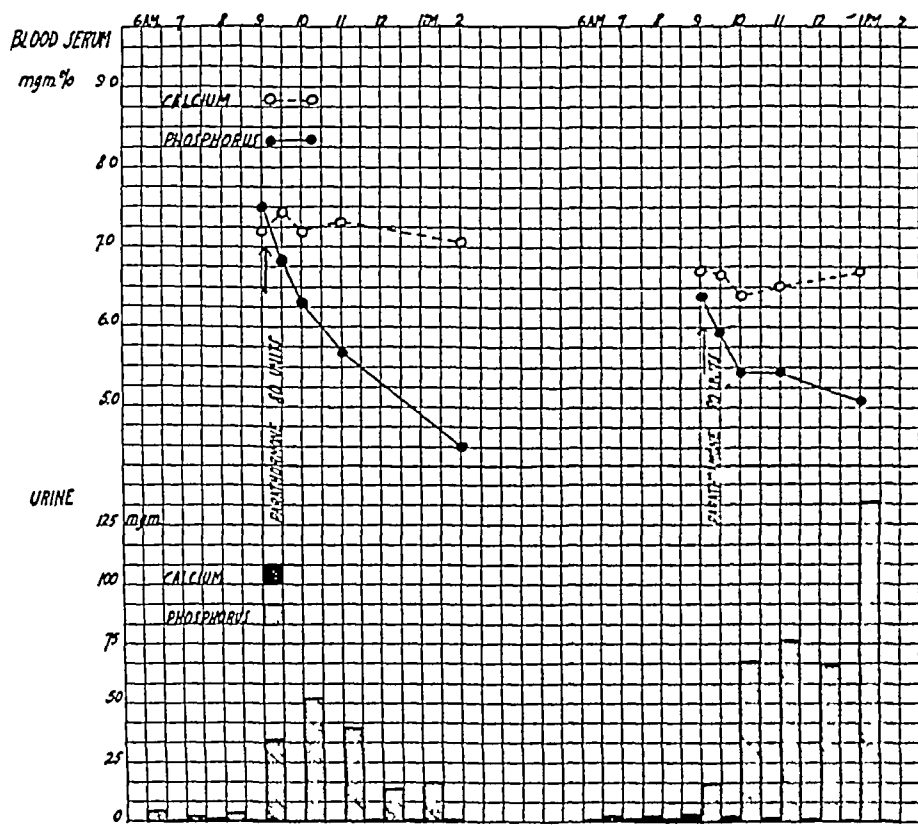


CHART 2 SERUM AND URINARY CALCIUM AND PHOSPHATE IN SUBJECT H C

would have blood volumes of approximately 2530 cc and 3860 cc respectively. Figures of 1800 cc and 2500 cc for the *plasma* volumes were taken as maximum figures, which would be well above any probable variation. The amount of phosphorus lost from the serum, assuming the maximum plasma volumes, was then calculated from the observed fall in serum phosphorus. In Table 1 it is seen that, even assuming the maximum plasma volumes, there is sufficient phosphorus in the urine, during the first hour after the injection of parathormone, to account for the decrease of phosphorus in the serum.

TABLE 1  
Comparison of serum phosphate and urinary phosphate excretion

Experi- ment number	Subject	Surface area	Average blood volume per sq. in.†	Blood volume esti- mated from surface area	Maxi- mum probable plasma volume‡	Fall in serum inor- ganic phos- phorus 1st hour	Phos- phorus lost assuming maximum plasma volume	Phos- phorus found in urine 1st hour after para- thormone
		sq. m.	cc.	cc.	cc.	mgm. per 100 cc.	mgm.	mgm.
1	A C	1.1	2300*	2530	1800	0.45	8.1	11.8
2	A C	1.1	2300	2530	1800	1.00	18.0	18.7
3	H C	1.6	2300	3680	2500	1.3	32.5	33.6
4*	H C	1.6	2300	3680	2500	1.0	25.0	83.9

\* First two hours taken, as first hour urine was collected few minutes early

† From Chang and Harrop (7)

‡ For maximum plasma volume approximate figures were chosen which would be well above any probable variations. Plasma volume  $\times$  observed fall in inorganic phosphorus = mgm. phosphorus lost from plasma

TABLE 2  
Ultrafiltration

Subject	Sample	Inorganic phos- phorus in serum corrected for protein	Inorganic phos- phorus in filtrate	Filtrability
		mgm. per 100 cc.	mgm. per 100 cc.	per cent
A C	1	8.24	8.2	100
	2*	8.05	7.9	98
H C	1	7.66	7.5	97
	2	6.9	6.8	98

\* One half hour after parathormone.

The behavior of the serum calcium shows an interesting contrast to that of the serum phosphorus. Whereas the change in the latter was immediately detected, the alteration in serum calcium level was delayed. In Experiments 1 and 2 the delay was slight, though definite, whereas in Experiments 3 and 4 the lag in calcium change was more than 5 and 4 hours respectively. In these experiments blood specimens taken 24 hours after the parathormone showed the usual rise in serum calcium. Such observations offer stronger evidence than we have before obtained for the hypothesis that the changes in phosphorus metabolism following parathyroid administration are primary and the alterations in calcium metabolism are secondary.

As an explanation of the phosphorus diuresis following the injection of parathormone, any hypothesis that the phosphorus is forced out of the serum in some way as an adjustment to a rising serum calcium is untenable in those instances in which the serum calcium did not rise for 4 hours.



One is again left with the hypothesis that the phosphorus changes occur as the first effect of the injection of parathormone

To explain the outpouring of phosphorus in the urine in combination with a falling serum inorganic phosphorus, two possibilities suggest themselves. The first is that the hormone might convert a grossly nonfiltrable form of phosphorus into a filtrable state so that it would more readily pass the kidney. By using Grollman's technique, however, it was found (Table 2) that from 97 to 100 per cent of the inorganic phosphorus of the serum of these two subjects was readily filtrable before parathormone was given.

There remains then the hypothesis that parathyroid extract lowers the renal threshold for phosphorus, although the term renal threshold still wants an accurate definition. In this case the sequence of events leading to the eventual rise of serum calcium and the calcium diuresis would appear to be (a) Lowering of renal threshold for phosphorus (b) Rise of phosphorus excretion in urine (c) Fall of serum inorganic phosphorus (d) Rise of serum calcium in adjustment to (c) (e) Rise of urinary calcium excretion.

#### SUMMARY

1 In four experiments, as previously observed, the first effect of parathormone injection in two patients with hypoparathyroidism was an immediate outpouring of phosphorus in the urine and a fall of serum phosphorus.

2 By taking blood specimens at very short intervals it was found that the fall in serum phosphorus preceded the rise in serum calcium.

3 The hypothesis that the parathyroid hormone affects primarily the phosphorus metabolism is thereby strongly supported.

4 By direct ultrafiltration experiments the serum phosphorus was shown to be 97 to 100 per cent filtrable before parathormone was given.

5 To explain the initial phosphorus diuresis, one is left with the suggestion that parathormone lowers the renal threshold for phosphorus.

The author wishes to acknowledge his indebtedness to Miss M. Struve for her very careful work in supervising the metabolism routine and for making the charts.

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# VASODILATATION IN THE LOWER EXTREMITIES IN RESPONSE TO IMMERSING THE FOREARMS IN WARM WATER

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Several tests have been proposed, and are now in general use, for estimating the grade of organic occlusion existing in the blood vessels of an extremity. The principle underlying these various methods is similar. The surface temperature of the distal portion of the cool, exposed extremity is measured thermo-electrically. Dilatation of the peripheral vessels is then produced and the coincident rise in surface temperature is recorded. The level to which the temperature rises with complete vasodilatation has been determined in individuals with normal peripheral circulation. If the surface temperature fails to rise to this normal level, the arteries supplying the part are regarded as being unable to dilate due to organic changes in their walls.

One of the earliest methods of producing vasodilatation in the extremities in man was suggested by Brown (1) and consists in producing fever by the intravenous injection of typhoid vaccine. Morton and Scott (2) induced vasodilatation in the lower limbs by means of spinal anesthesia, and later proposed the use of general anesthesia, ether, nitrous oxide or ethylene (3), for the same purpose. Injection of peripheral nerves with novocaine has also been used to produce local vasodilatation by White (4), Lewis (5), and Scott and Morton (6). Still more recently Lewis and Pickering (7) studied the vasodilatation in the hands produced by warming the body. The subject was seated in a small hot air chamber with the head and hands projecting into a cool room. Heating the air in the chamber to a temperature of 46° C or more was followed by a rise in the skin temperature of the hands. They suggest that the method may also be used to produce vasodilatation in the feet.

The following report describes the effects which warming one or two extremities exerts on the skin temperature of the other extremities, with special emphasis on the vasodilatation in the feet produced by immersing the forearms in warm water. The mechanism by which this vasodilatation is induced has been studied in normal subjects. The simplicity of the method and apparatus, as well as the constancy of the reaction in normal subjects, suggests that the procedure may have a certain value in the

diagnosis of peripheral vascular diseases Only three illustrative cases are described, a more comprehensive clinical study will appear later

#### METHOD

The observations on normal subjects have been carried out at room temperatures varying from 11.4 to 21.5° C The subjects were seated in a chair with the feet resting on a stool about a foot from the floor Room temperature was read from a mercury thermometer suspended in the air near the exposed extremities The surface temperatures of the toes were determined thermo-electrically in the usual manner The thermal junctions were placed in contact with the skin on the dorsal surface of the distal phalanx just proximal to the base of the nail In some instances the junctions were bare and in others a single layer of surgeon's plaster covered the junction without modifying the results significantly Skin temperatures were measured at 2 minute intervals while the exposed extremities cooled, and when the skin temperature was sufficiently low (usually 25° C or less) the forearms were immersed in warm water (43 to 45° C) to a point just above the elbows For this purpose ordinary white enamel arm baths, 52 cm long, 20 cm wide and 15 cm deep, were entirely satisfactory The temperature of the water was kept between 43 and 45° C by means of a small electrical heating coil placed beneath each bath While the forearms were immersed in warm water skin temperature and room temperature were read every minute

While the primary object of these experiments was to study vasodilatation in the lower extremities, a certain number of observations were made upon the vasodilatation in the hands produced by immersing the legs in warm water In these experiments the method was essentially the same The hands were exposed until cool, then the legs were immersed in warm water to a level midway between the ankle and knee The two receptacles used as foot baths were metal cans, open at the top, 24 cm square and 35 cm deep At the bottom of each can a small wooden frame weighted with lead prevented the sole of the foot from coming into contact with the metal The bath was maintained at a temperature of 43 to 45° C by means of a small heating coil beneath each can

#### OBSERVATIONS

##### *1 General vasodilator effects of immersing one or two extremities in warm water*

The typical rise in the skin temperature of the feet observed when the hands and forearms are immersed in warm water is shown in Figure 1 The subject (G) had been in a cool room (16 to 17° C) for two hours The coat was removed and the shirt sleeves were rolled up above the elbows The subject was seated, his feet were supported about 15 inches from the floor and exposed to the room air Thermal junctions

were placed on the right first and second and the left first toes. During the first 20 minutes of exposure the temperatures of the toes fell from between 19 and 20° C to between 17 and 18° C. Both arms were then immersed in warm water (44 to 45.5° C). Four minutes later the subject felt warm, one minute after that the face was flushed and the forehead slightly moist with perspiration. Ten minutes after the forearms had been immersed the subject was perspiring profusely. The left first toe started to become warm ten minutes after the forearms had been immersed and was followed three minutes later by the right first and second toes.

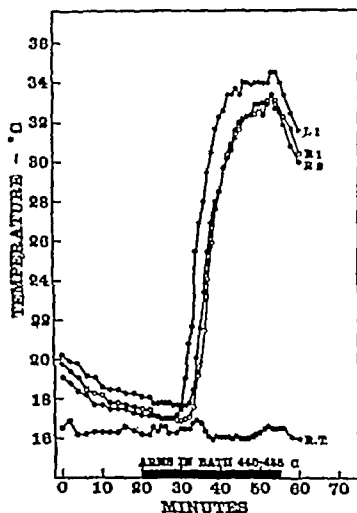


FIG 1 SHOWING VASODILATATION IN THE TOES PRODUCED BY IMMERSING BOTH FOREARMS IN WARM WATER

L1, left first toe, R1, R2, right first and second toes, R.T., room temperature.

Thereafter the skin temperature of all the toes rose rapidly, reaching in a few minutes maximum levels of 33 to 34° C. The forearms were removed from the warm water 35 minutes after being immersed. This was followed by a rapid fall in the surface temperature of the toes.

The normal vasodilator response in the hands following immersion of the legs in warm water is shown in Figure 2. The subject, La, entered the cool room (14° C) after having been in a warm environment for several hours. Coat, shoes and socks were removed, and the shirt sleeves were rolled above the elbows. The subject was seated in a chair with his hands resting palm downward on a table. Thermal junctions were placed over the dorsum of the middle phalanx of the left third and right

fourth fingers and on the dorsum of the right hand. In this observation the rectal temperature was also recorded by means of a fourth thermal junction placed opposite the opening of a small catheter introduced into the rectum for a distance of 10 cm. Surface temperatures were then recorded for a preliminary period of 73 minutes. During the first 30 minutes the surface temperatures of the hand and fingers fell slowly. By the 30th minute the subject felt definitely chilly, and from then on the

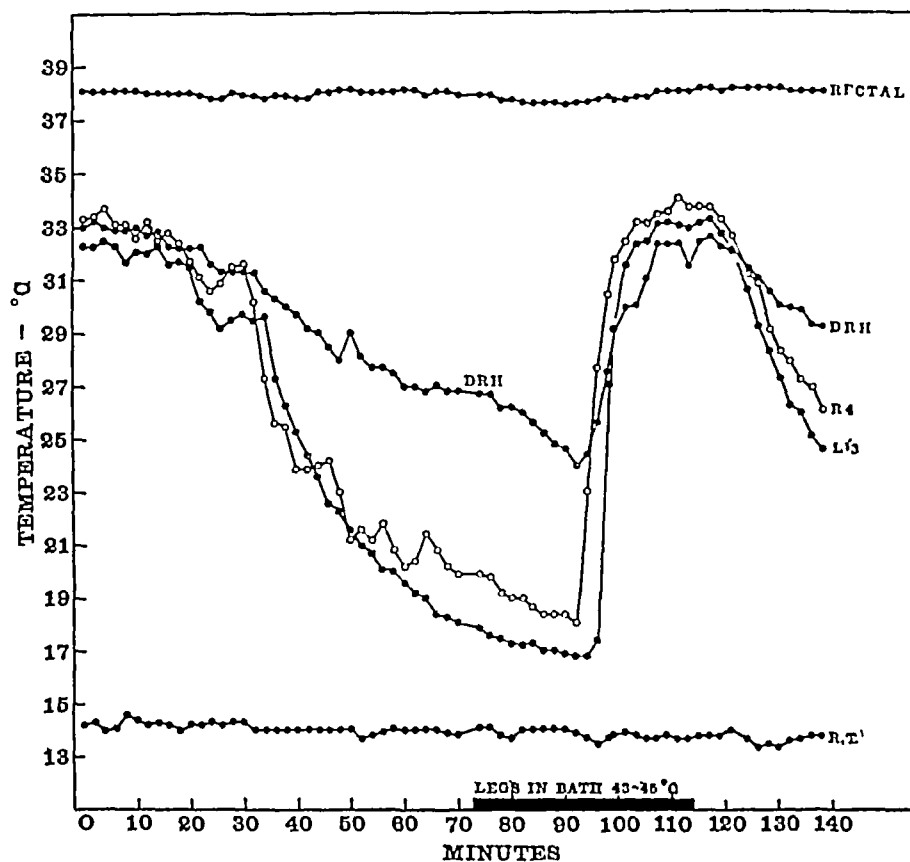


FIG 2 SHOWING VASODILATATION IN THE FINGERS PRODUCED BY IMMERSING BOTH LOWER LEGS IN WARM WATER. RECTAL TEMPERATURE IS SHOWN AT TOP OF CHART

DRH, dorsum right hand, R4, right fourth finger, L3, left third finger, R.T., room temperature

surface temperatures fell more rapidly. At the 73d minute both lower legs were immersed in water at a temperature of 43 to 45° C. Ten minutes after immersion of the legs the subject still felt definitely chilly and gooseflesh was present over both the forearms. Six minutes later the subject was comfortably warm and the forehead was moist to the touch. After the legs had been immersed 19 minutes the temperatures of the fingers and hand began to rise and in a few minutes had exceeded 32° C.

After the legs had been removed from the baths and dried, the temperatures of the hand and fingers began to fall and the subject again felt chilly

It can be seen in Figure 2 that the temperature of the dorsum of the hand, although initially higher than that of the fingers, did not rise as rapidly nor to as high a level as did the digital temperature Lewis and Pickering (7) have demonstrated that the tips of the fingers show the earliest and greatest rise in surface temperature when vasodilatation is induced by warming the body These authors have suggested that the difference in temperature response is due to the presence in the distal phalanges of arteriovenous anastomoses, the importance of which Grant and Bland (8) have demonstrated

Warming a sufficient area of one limb also produces vasodilatation in the other limbs Figure 3 shows the effect of warming the lower half of

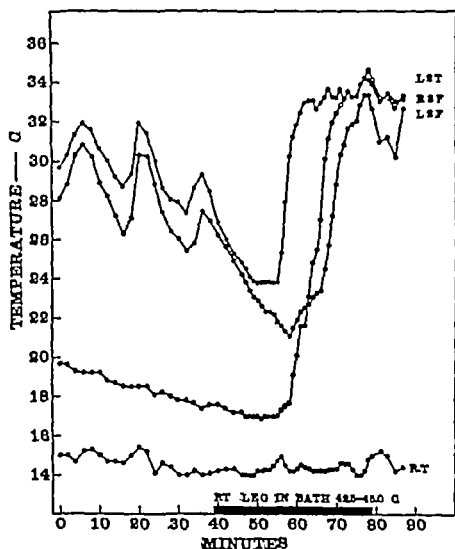


FIG 3 SHOWING VASODILATOR EFFECT OF IMMERSING THE RIGHT LOWER LEG IN WARM WATER

L2T, left second toe R3F, right third finger L2F, left second finger RT, room temperature.

the right leg upon the surface temperatures of the other three extremities During the control period of 39 minutes the temperature of the left second toe, which was already at a low level ( $19.7^{\circ}\text{C}$ ), fell steadily The



temperatures of the fingers fluctuated about a high level at the start of the observation but finally began to fall as the subject became definitely chilly. At the 39th minute the right lower leg was immersed in a bath at a temperature of 42.5 to 45° C. Sixteen minutes after the leg was immersed the subject still felt definitely chilly. Three minutes later the face was flushed and by the 60th minute, 21 minutes after immersion of the leg, the subject felt comfortably warm. Shortly afterward the temperatures of all three extremities had risen well above 32° C. Immersion of one forearm in hot water produces a similar rise of skin temperature in the opposite fingers and in the toes (Figure 4)



FIG 4 SHOWING VASODILATOR EFFECT OF IMMERSING THE LEFT FOREARM IN WARM WATER

R3F, right third finger, R2T, right second toe, RT, room temperature

In normal subjects warming two extremities in the manner described has always produced complete vasodilatation in the digits of the other two extremities. Warming one extremity, however, does not always produce complete vasodilatation in the other three extremities. Such partial or incomplete responses have been observed when one upper extremity has been warmed only as far as the wrist, or when one lower extremity has been warmed only as far as the ankle.

## 2 The time relations of the vasodilator response in the lower extremities of normal subjects

Table I summarizes the results of immersing the hands and forearms of six normal subjects in warm water. The room temperatures varied between 11.4 and 21.5° C. The temperatures of the toes before the rise ranged from 16.9 to 28.5° C. In all but the last observation the feet were cooled by exposing them to room air. After such spontaneous cooling the skin temperature began to rise within 3 to 15 minutes after the forearms were immersed, reaching 32° C within 7 to 29 minutes after the beginning of the immersion. Usually 3 or 4 toes were observed in each subject and in every instance but one the temperature finally rose to values exceeding 32° C. In this one exception after the forearms had been immersed for 35 minutes the temperature of one toe was 31.6 while the others had reached 32.1, 33.1 and 33.6° C. The thermocouple later proved to be defective so that this exception may not be a real one. The skin temperature of the digits always rose above that level which Morton and Scott (3) regard as the minimum normal response to spinal anesthesia (i.e. 31.5° C).

TABLE I  
*The vasodilatation produced in the feet by warming the arms*

Subject	Room temperature	Toe	Temperature of toe before rise	Time from immersion to start of rise	Time to reach 32° C.	Maximum temperature
	C		C	minutes	minutes	C
G	16.5	R1	16.9	12	25	33.2
		R2	17.7	13	26	33.4
		L1	17.0	10	20	34.5
G	20.0	R1	21.3	14	20	35.4
		L1	20.7	12	20	35.0
		L3	19.9	12	20	35.2
T	11.4	L2	19.0	5	11	33.2
B	18.4	L3	22.4	9	15	33.5
		L1	23.8	9	13	33.8
		R3	22.8	8	14	35.1
		R1	28.5	3	7	35.8
L1	19.1	L1	20.4	8	17	32.1
		L3	19.9	15	29	33.1
		R1	20.3	6	12	33.6
		R3	19.5	13	—	31.6
M	19.0	L1	24.0	12	17	33.8
		R1	23.8	9	14	34.7
		R3	23.2	10	17	34.3
La *	21.5	R2	21.9	23	39	33.8

\* Feet previously immersed in cold water (22° C) for 22 minutes

A conspicuously delayed reaction was observed in subject La (Table I) after the feet had been cooled by immersing them in water at 22° C for 22 minutes. In spite of the delay the temperature finally reached 33.8° C. It is certain that the delay in this instance was not due to any real abnormality of circulation since the same subject on two occasions reacted within the usual time (Table II) when the feet had cooled spontaneously. On account of this delayed response the use of a cold bath to reduce the temperatures of the extremities was avoided in all subsequent observations.

TABLE II  
*Effect of clothing on time of rise in skin temperature*

	Subject G				Subject La			
	Room temperature	Temperature of toes before rise	Time from immersion to start of rise	Time to reach 32° C	Room temperature	Temperature of toes before rise	Time from immersion to start of rise	Time to reach 32° C.
	° C	° C	minutes	minutes	° C	° C	minutes	minutes
Without blankets	20.0	19.9	12	20	16.0	17.2	7	22
		20.7	12	20		17.9	7	13
		21.3	14	20		20.1	6	9
With blankets	19.5	18.9	9	14	16.0	18.1	14	29
		19.2	10	15		18.3	12	23
		20.1	9	14		20.4	7	15

The time elapsing between immersion of the forearms in warm water and the appearance of vasodilatation in the feet is probably affected by a number of factors, some of which are not easy to evaluate. A few of the more likely factors have been investigated. Lewis and Pickering (7) have already shown that the time interval between warming the body and the rise of temperature of the fingers "is influenced by the initial temperature of the extremities, being delayed by coldness." Similar results have been observed in the temperature changes produced by vasodilatation in the feet (Table I, subject B). As the initial temperatures of the extremities will vary widely in different observations, it is doubtful whether any significance can be attached to the variations in the time elapsing between immersion and the beginning of the temperature rise in the other extremities.

The observations upon normal subjects have been carried out at both high and low room temperatures. The temperature of the room air alone appears to have little influence upon the time at which vasodilatation begins (Table I). Thus on one occasion with the room temperature between 11 and 12° C the temperature of the toe (initially 19.0° C) began to rise 5 minutes after immersion of the arms, and was above 32° C in 11

minutes The subject on this occasion was warmly clad Another subject, at a room temperature of  $20.0^{\circ}\text{C}$ , did not show the response until 12 to 14 minutes after the forearms were immersed

As this type of vasodilatation apparently results from warming the body, it was thought that the clothing of the subject might influence the time relations of the response, and that in a lightly clad subject exposed to a very low room temperature the response might be delayed or even absent Two observations were carried out with subjects clad only in short trunks and sleeveless cotton jersey at room temperatures of  $19.5$  to  $20^{\circ}\text{C}$  and  $16^{\circ}\text{C}$  respectively In both instances after the forearms were immersed in warm water the temperatures of the toes began to rise at the usual time and reached  $32^{\circ}\text{C}$  within 22 minutes (Table II) The observations were then repeated under similar conditions with the exception that while the arms were immersed in hot water the subject was enveloped from neck to ankles in two heavy blankets In one subject (G) the temperature of the toes rose more promptly while in the other subject (La) the response appeared later (Table II) Thus the manner in which the subject is clad does not appear to exert any conspicuous effect upon the response at room temperatures of 20 and  $16^{\circ}\text{C}$ .

### *3 The mechanism by which warming one forearm produces vasodilatation in the opposite hand*

There are two possible mechanisms by which this type of vasodilatation may be initiated The response may be due to sensory impulses from the limbs immersed in hot water, or it may be due to the heat carried into the body by the venous blood, returning from the warmed limbs Pickering (9) has already shown that the central mechanism controlling vasomotor tone in the extremities is affected by changes in body temperature amounting to less than  $0.1^{\circ}\text{C}$

In 1911, G N Stewart (10) reported certain calorimetric studies on the rate of blood flow in the hands He found in two experiments on normal subjects that immersing one hand in warm water increased the blood flow through the opposite hand In one instance a measurable increase in blood flow was observed two minutes, and in the other five minutes, after the opposite hand was immersed in warm water ( $43^{\circ}\text{C}$ ) In the first observation the temperature of the water in the calorimeter was  $30^{\circ}\text{C}$  and in the second  $24^{\circ}\text{C}$  Stewart (10) regarded the phenomenon as "reflex vasomotor excitation," and attributed a part of the delay in the response to the fact that any change in the temperature of the water in the calorimeter must necessarily "lag somewhat behind the corresponding change in blood flow"

In our observations the time elapsing between immersion of the limbs in hot water and the rise of temperature in the other limbs amounted to between 5 and 15 minutes This delay in the appearance of the response

suggests that this type of vasodilatation is not due to a simple nervous reflex. The general feeling of warmth which precedes the rise in digital skin temperature, and the generalized perspiration, suggest, moreover, that the vasodilatation results from an increase in body warmth.

More conclusive evidence that in our observations vasodilatation was not produced by afferent nerve impulses but was dependent upon the flow of blood through the limbs immersed in hot water was obtained in the following manner. One hand was exposed to an air temperature of 18.5 to 20.0° C. A pneumatic cuff was wrapped about the opposite upper arm and inflated suddenly from a reservoir to a pressure well above the systolic level. The forearm, with the circulation cut off, was immersed in warm water (42.5 to 44.6° C) and the temperatures of the fingers of the opposite hand were recorded every minute. After 15 minutes the blood vessels of the warmed forearm were released for a period of two minutes, when blood flow was again stopped. After a second period of occlusion for 10 minutes the circulation was released again for one minute and then occluded once more for a final period of 10 minutes. The arm was kept in the bath until the customary response in the fingers of the opposite hand had occurred (Figure 5). This procedure was carried out in two

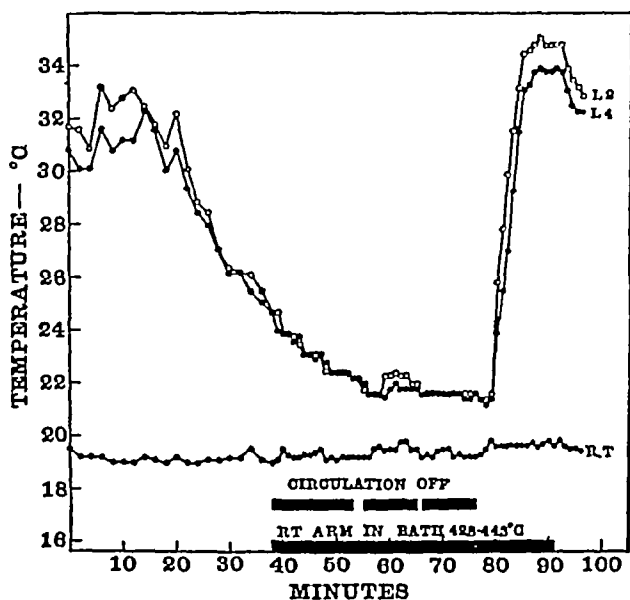


FIG 5 SHOWING THE DELAYED VASODILATOR RESPONSE PRODUCED BY OCCLUDING CIRCULATION IN THE IMMERSSED FOREARM (RIGHT)

L2, left second finger, L4, left fourth finger, R T, room temperature

observations, while in the third the occlusion was divided into two periods of 20 minutes and 15 minutes, with an interval of 3 minutes during which the circulation was released. Thus in all three observations the circulation to the arm in the bath was completely occluded for a total period of

35 minutes. In all three experiments the temperature of the fingers of the opposite hand failed to rise until some minutes after the circulation had been finally reestablished in the immersed forearm.

The results of these three observations are summarized in Table III (a), for comparison with the results of six control observations in Table III (b). When the blood vessels of the arm in the bath were occluded for

TABLE III  
*Effect of occluding the circulation of the immersed arm*

(a) With occlusion of the circulation of the immersed arm for 35 minutes						(b) Without occlusion					
Subject	Room temperature	Temperature of fingers before rise	Time from immersion to start of rise	Time to reach 32° C.	Bath temperature	Subject	Room temperature	Temperature of fingers before rise	Time from immersion to start of rise	Time to reach 32° C.	Bath temperature
La	C 18.5	C 19.9	min 44	min 48	C 42.5 to 43.8	G	C 17.0	C 20.2	min 10	min 16	C 42.8 to 43.5
		19.6	44	48				19.8	10	16	
La	19.5	20.8	45	51	43.0 to 44.6	G	19.5	21.1	9	14	42.9 to 43.6
		20.7	44	50				21.3	8	11	
G	19.5	21.2	42	47	42.8 to 44.3	La	16.5	20.3	5	13	42.7 to 43.4
		21.4	42	46				20.7	5	10	
						La	20.5	21.2	6	9	43.7 to 45.0
								21.5	5	11	
						La	15.5	22.9	6	10	44.0 to 45.0
						J	14.5	23.8	10	16	43.8 to 45.3

35 minutes the temperature response in the opposite hand never began before the forty second minute. In the six control observations, in which one forearm was immersed without occlusion, the longest period before the temperature response began was only 10 minutes. Cutting off the circulation in the immersed forearm, therefore, conspicuously delayed the appearance of vasodilatation in the opposite hand. This effect cannot be attributed to interference with the function of the sensory nerves of the immersed limb. It is obvious that the only afferent nerves which could possibly be involved are those concerned with the perception of warmth.

When the circulation is occluded a bath temperature of 43.0 to 43.5° C produces an almost intolerable sensation of heat. In addition, heat sensation was tested at frequent intervals by pouring a small amount of water at a temperature of 47 to 48° C into the bath near the fingers and forearm. This water, even though mixed with the water in the bath, invariably felt disagreeably hot to the subject. According to this test, heat sensation was present and very slightly or not at all diminished throughout the entire period of occlusion. This agrees with the work of Lewis, Pickering, and Rothschild (11), who reported that heat sensation is still acute after the circulation of the forearms (at 30° C) has been occluded for 35 minutes without interruption.

The delayed response accompanying occlusion of the circulation, therefore, presents strong evidence against the hypothesis that this type of vasodilatation results from afferent nerve impulses originating in the immersed limb. It seems clear that the vasodilator response depends on the return of warmed blood from the immersed extremity.<sup>1</sup> Rectal temperature was measured thermo-electrically on three occasions in order to learn whether vasodilatation in the extremities was associated with large changes in body temperature. In observation 4 (Figure 2) the rectal temperature fell very slightly during the preliminary control period, when the subject felt definitely chilly, and rose again slightly, shortly after the legs had been immersed in hot water. The rise in rectal temperature followed the appearance of subjective sensations of warmth. On another occasion the changes observed were similar in direction but of slightly greater magnitude (0.6° C). In a third observation the greatest variation observed was 0.1° C. Since this type of vasodilatation is dependent upon the return of warmed blood from the immersed extremity, it follows that the central nervous mechanism, which responds to warming of the body by diminishing vasoconstrictor tone in the extremities, is sensitive to very small changes in body temperature, otherwise, the rise in rectal temperature would have been greater.

The effector mechanism of the vasodilator response to warming the body has already been studied by Lewis and Pickering (7), who showed that the response is absent in the sympathectomized extremity. They concluded that the effector mechanism lies purely in the sympathetic nerves to the limb and is not antidromic.

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<sup>1</sup> More recently this point was tested further in a patient with paraplegia due to transverse myelitis. Pain and temperature sensations were lost anteriorly below the level of the seventh dorsal segment and posteriorly below the level of the tenth dorsal segment. The anesthetic legs were immersed in warm water at a temperature of 43° C. Twelve minutes later the subject felt warm and began to perspire. The temperatures of the fingers, originally 23.3 to 23.6° C, began to rise fifteen minutes after the legs were immersed, exceeding 32° C by the twenty-fifth minute after immersion. In this instance, the vasodilatation in the fingers occurred without sensation of warmth in the immersed extremities. This provides additional evidence that the type of vasodilatation here described is due to the return of warmed blood from the immersed limbs.

## CLINICAL STUDIES

In ten observations on the spontaneously cool lower extremities of six normal subjects the digital skin temperature began to rise within 15 minutes after the forearms were immersed in warm water. The skin temperature in all but one of these observations exceeded  $32^{\circ}\text{C}$  by the 29th minute. The amount of vasodilatation produced by this relatively simple procedure is apparently as great as the vasodilatation produced by spinal anesthesia or nerve block. The method was used, therefore, in studying a series of patients with clinical evidence of inadequate circulation in the lower extremities.

Room temperature was usually kept between  $18^{\circ}\text{C}$  and  $20^{\circ}\text{C}$ . The lower extremities were permitted to cool spontaneously and then the forearms were immersed in warm water for 35 minutes, a period definitely longer than that required for a complete response in normal subjects. Abnormal findings were checked by other methods of examination. The following cases exemplify the clinical usefulness of this simple procedure, when it is necessary to distinguish between organic occlusion and spasm of the peripheral blood vessels in the lower extremities.

*Case 1* B S, an Austrian Hebrew male, aged 58, complained of pain and swelling in the right lower leg, accompanied by symptoms of intermittent claudication in the right calf. The symptoms had begun 8 months prior to examination and had grown progressively worse. At night the right foot habitually became cold and painful even though protected by blankets or a warmed cradle. No history of migratory phlebitis could be elicited. The patient smoked about 10 cigarettes per day.

On examination the systolic blood pressure was 155 and the diastolic 90, the brachial and radial arteries were moderately sclerotic. The left foot was normal in appearance while the right foot showed marked rubor and, when dependent, definite cyanosis of the sole and of the distal phalanges of the toes. The distal portion of the foot blanched conspicuously when elevated and the return of color was delayed. No pulsation could be felt in the dorsalis pedis or the posterior tibial arteries of either foot. X ray examination revealed a moderate and equal grade of calcification of the arteries of both feet and both lower legs. There was marked osteoporosis of all the bones of the right foot.

The patient's feet were exposed to room air at a temperature of  $18$  to  $19^{\circ}\text{C}$ . He was clothed in a shirt, heavy underwear and trousers. The lower extremities were exposed as far as the knees. The temperatures of the right and left great toes were fairly constant during the control period of 30 minutes (Figure 6, lower chart).

Immersing the arms in water at  $44.5$  to  $45^{\circ}\text{C}$  for 35 minutes caused the temperature of the left great toe to rise slowly to a maximum of  $27.7^{\circ}\text{C}$  but the temperature of the right great toe did not change. These abnormal responses suggested the presence of slight organic obstruction to circulation in the left foot, and marked organic obstruction in the right foot.

The changes in skin temperature during spinal anesthesia confirmed these findings (Figure 6, upper chart). The patient, clad in hospital shirt and covered by a sheet, lay on a bed with the legs and feet exposed. Thermal



junctions were fixed to the right and left great toes, close to the bases of the nails, and skin temperatures were recorded for a preliminary period of 20 minutes. The room temperature varied between 18 and 19° C. Fifty mgm of neocaine dissolved in spinal fluid were injected into the spinal canal between

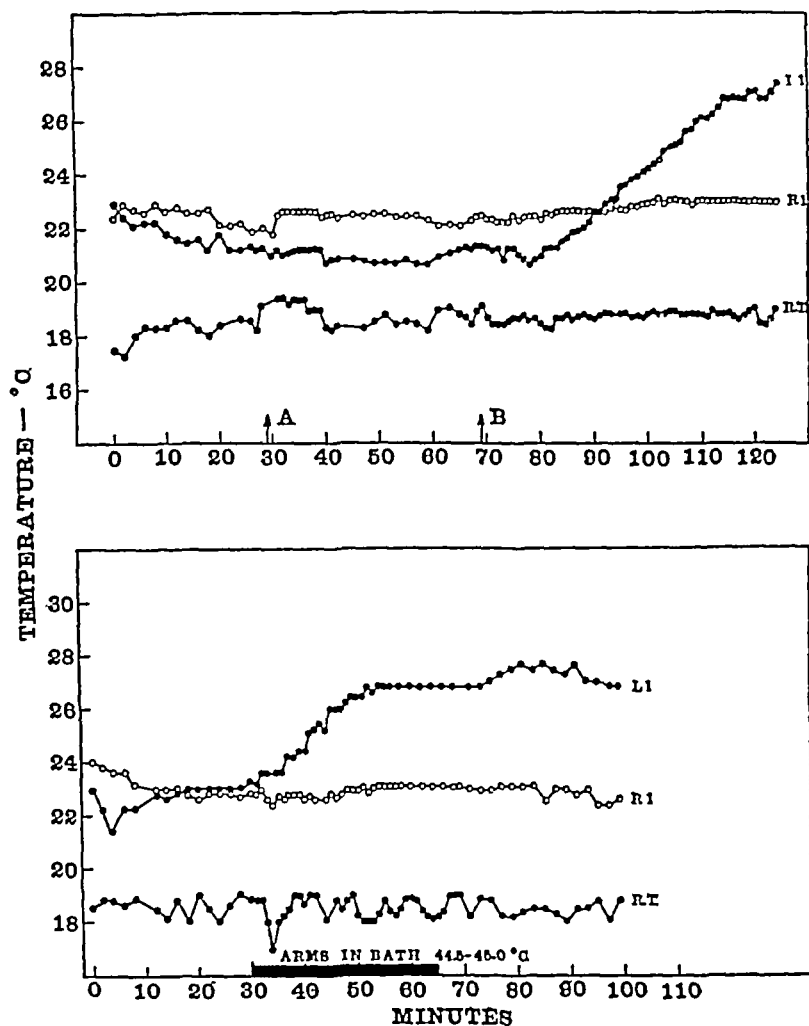


FIG 6 UPPER SHOWING CHANGES IN SKIN TEMPERATURE OF THE LEFT FIRST (L1) AND RIGHT FIRST (R1) TOES AFTER SPINAL ANESTHESIA IN A PATIENT WITH ARTERIOSCLEROSIS

A Fifty mgm neocaine injected into the spinal canal between 4th and 5th lumbar vertebrae B Fifty mgm neocaine injected into the spinal canal between 3d and 4th lumbar vertebrae RT, room temperature

LOWER SHOWING CHANGES IN SKIN TEMPERATURE OF SAME TOES DURING IMMERSION OF FOREARMS IN WARM WATER

the third and fourth lumbar vertebrae. The perineum became anesthetic, but sensation in the legs remained normal. At the 69th minute another 50 mgm of neocaine was injected into the spinal canal between the second and third lumbar vertebrae and thoroughly mixed with spinal fluid. By the 85th

minute the feet, legs and thighs were completely anesthetic. Subsequently, the temperature of the left first toe rose slowly and steadily to  $27.4^{\circ}\text{C}$  while the temperature of the right first toe did not change. The graphs in Figure 6 show that immersing the arms in warm water and spinal anesthesia affected the skin temperature similarly. In the left great toe the maximum temperature and the rate of the rise were similar in both procedures. The temperature of the right great toe did not change significantly in either case.

The surface temperature changes in this case indicate a high grade of organic obliteration in the blood vessels of the right foot, with mixed spasm and a mild grade of organic obliteration in the blood vessels of the left foot. The obliterative changes were probably due to arteriosclerosis but the patient was one of those in whom thrombo-angitis obliterans could not be definitely excluded by clinical examination.

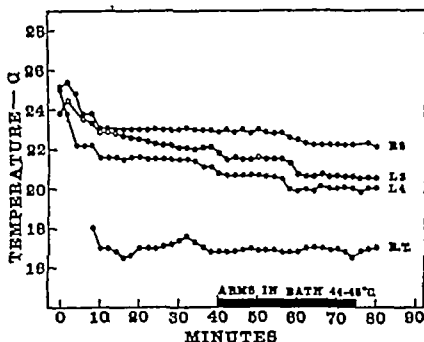


FIG 7 SHOWING SKIN TEMPERATURE OF THE RIGHT THIRD (R3) AND LEFT THIRD AND FOURTH (L3, L4) TOES, WHILE THE FOREARMS WERE IMMERSED IN WARM WATER

RT, room temperature. The patient had advanced thrombo-angitis obliterans involving both feet.

*Case 2* N,<sup>2</sup> a white male, aged 26, was admitted to the University Hospital on December 2, 1931, with a chief complaint of pain in the lower extremities. For two years the patient had suffered to an increasing degree from generalized pain in the lower legs; intermittent claudication had been present for eight months prior to admission. Discolored areas were first observed on the great toes about nine weeks before admission. The patient smoked approximately 20 cigarettes per day.

On examination his blood pressure was normal, there was no evidence of arteriosclerosis nor of diabetes. The skin of both feet showed purplish red discoloration with a spot of dry gangrene about 4 cm in diameter on the mesial surface of each great toe. X ray examination revealed no calcification of the vessels of the legs. The bones of the left foot and the lower end of the left tibia were considerably rarefied. The vasomotor index, determined by

<sup>2</sup> We wish to express our indebtedness to Dr. George P. Müller for his kindness in permitting us to include this case and to Dr. Lee Rademaker for the results of the vasomotor index test.

Dr Lee Rademaker, was zero for both feet. The patient improved somewhat under conservative treatment and was discharged December 9, 1931, with a diagnosis of thrombo-angitis obliterans affecting both lower extremities.

He was readmitted to the Hospital on March 19, 1932, with more extensive gangrene of both great toes. At this time it was necessary to remove the terminal phalanx of the left great toe and the entire right great toe.

The vasodilator response in the lower extremities was tested several days after operation. The patient was seated in bed with the feet exposed to an air temperature of  $17^{\circ}\text{C}$ . Small gauze dressings covered the sites of amputation. The four lateral toes of each foot were cyanosed and both feet were cold as far as the ankles. Thermal junctions were fixed to the dorsal surfaces of the distal phalanges of the right third and the left third and fourth toes. As shown in Figure 7 the skin temperature, originally about  $24$  to  $25^{\circ}\text{C}$  gradually fell, during the course of 40 minutes, to between  $20$  and  $23^{\circ}\text{C}$ .

The forearms were then immersed in water baths ( $44$  to  $45^{\circ}\text{C}$ ) for 35 minutes. Perspiration appeared on the patient's forehead four minutes after the forearms were immersed. As indicated in Figure 7 the surface temperature of the toes showed no rise but, on the contrary, a slow decline. The test was repeated later with a room temperature of  $21^{\circ}\text{C}$  and a similar result was obtained. In the latter observation the digital temperatures ranged from  $25.0$  to  $26.2^{\circ}\text{C}$  before immersion of the forearms and from  $24.0$  to  $25.4^{\circ}\text{C}$  at the end of the period of immersion.

The history and physical findings are typical of thrombo-angitis obliterans. Immersing the forearms in warm water produced no change in the skin temperature of the toes. This indicates the presence of a very severe grade of organic occlusion of the vessels of both feet,—a finding which is in agreement with the results of the vasomotor index test.

*Case 3* R, a white female, aged 24, complained of pain in all four extremities. It had occurred during the previous 7 or 8 years, generally when the patient was fatigued, and was more severe in the lower extremities. There was no history of intermittent claudication, and the pain had no direct relation to exercise. It was described as a dull ache, and occurred as readily in a warm room as in a cold one. The patient had never noticed blue fingers or toes, but numbness was often experienced. The pain was not typical of any of the usually described vascular disorders. She had never had frost bite or chilblains.

On examination in a warm room ( $22^{\circ}\text{C}$ ) the toes were definitely cold, but only slightly, if at all, cyanotic. Neither the dorsalis pedis nor the posterior tibial arteries could be palpated in either foot. This was probably due to brawny edema of the ankles and dorsa of both feet. The edema did not pit on pressure but according to the patient, it increased during the day and disappeared during the night. Both radial and ulnar pulses were normal, the systolic blood pressure was 118 and the diastolic, 88. The patient was examined in order to decide whether there was any structural abnormality impeding blood flow in the lower extremities.

Immersing the feet for 20 minutes in a bath at  $15^{\circ}\text{C}$  failed to produce the spasm typical of Raynaud's disease. The feet were then dried and exposed to the room air at a temperature of  $21$  to  $22^{\circ}\text{C}$ . Thirty minutes later both forearms were immersed in water at a temperature of  $44^{\circ}\text{C}$ . Eleven minutes after the forearms were immersed the surface temperatures of the first toes of both feet began to rise and 22 minutes after immersion were well above  $32^{\circ}\text{C}$ .

The vasodilator response in the lower extremities of this patient was entirely normal, despite the fact that it was impossible to feel pulsation in

the arteries of either foot. This case report illustrates the value of the procedure in differentiating diminished blood flow arising from spasm and from structural disease of the blood vessels

#### DISCUSSION

The method which has just been described has certain advantages over the more complicated procedures by which vasodilatation may be produced. The test can be carried out with equal facility in patients whether ambulatory or in bed. It has been found particularly useful in those cases in which the use of spinal or general anesthesia was contraindicated.

There are two conditions, however, in which vascular spasm depends to a certain extent on the direct local effects exerted on the peripheral blood vessels by cold. Lewis (5) has shown that the arterial spasm of Raynaud's disease may be induced by cold even when vasoconstrictor impulses are removed by nerve block or by sympathetic ganglionectomy. In patients showing typical acrocyanosis arteriolar spasm is apparently induced similarly by cold (Lewis and Landis (12)). It is conceivable that in these two conditions the mere removal of vasoconstrictor impulses to the cooled extremity might not relax a vascular spasm stimulated by local cold. Scott and Morton (6) have stated that in certain cases of Raynaud's disease blocking the posterior tibial nerve produced only delayed or partial elevation of skin temperature. In two cases of acrocyanosis of the feet we found that immersing the forearms in warm water may fail to relax the arteriolar spasm in the lower extremities though the response, when it occurs, is normal in type. A full description of these patients will be published later. Lewis and Pickering (7) found also in one case of acrocyanosis of the hands that warming the body produced a response which, once initiated, was of the normal type.

The vasodilator response in the lower extremities produced by immersing the forearms in warm water (43 to 45° C) for 35 minutes has, except in acrocyanosis, been practically identical with vasodilatation produced by other procedures such as spinal anesthesia, nerve block, or the injection of typhoid vaccine. If the digital temperature rises above 32° C organic occlusion of the blood vessels is definitely excluded. If the digital temperature fails to rise, or rising fails to reach 32° C, the presence of organic obstruction is indicated, but this conclusion should probably be verified by some other method of examination. The simplicity of the test favors its use as a preliminary procedure in the study of cases showing clinical evidence of peripheral vascular disease.

#### SUMMARY

1. In normal subjects immersing the forearms in warm water (43 to 45° C) produces vasodilatation in the lower extremities. In ten observations on the spontaneously cool extremities of six normal subjects the

rise in digital skin temperature began within 15 minutes after immersing the forearms in water. The skin temperature in all but one of these observations exceeded  $32^{\circ}\text{C}$  by the twenty-ninth minute.

2 The immersion of one forearm or one leg in warm water produces vasodilatation in the other extremities.

3 This type of vasodilatation apparently depends upon the return of warmed blood from the immersed extremity.

4 The rise in rectal temperature produced by immersing two limbs in warm water ranged in three experiments from  $0.1^{\circ}\text{C}$  to  $0.6^{\circ}\text{C}$ .

5 Three cases illustrating vasodilator responses in certain types of vascular disorders of the extremities are reported. With this method of producing vasodilatation, if the digital temperature rises above  $32^{\circ}\text{C}$  organic occlusion of the vessels supplying the extremity may be excluded.

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# THE EFFECT OF NASO-PHARYNGEAL OPERATIONS ON RENAL FUNCTION

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The operations, tonsillectomy, adenoidectomy, and tooth extraction, are among the most practised therapeutic procedures in Bright's disease. Whether patients suffering from Bright's disease are able, without detriment to their kidneys, to undergo such operations, and whether the operations are likely to affect the subsequent course of the renal disease, are questions which form the object of the present analysis of results. It is based on data from the records of the nephritic clinic of the Hospital of the Rockefeller Institute collected over a period of several years.

Tonsillectomies and adenoidectomies were all performed by Dr Stuart Craig under gas-oxygen-ether anesthesia, and extractions of teeth by Dr Frank Wynn using procaine anesthesia. The patients were under the immediate care of Drs MacKay, Johnston, McIntosh, Moore, Kirk, and Alving.

The cases are classified according to the modification of Volhard and Fahr's classification made by Addis, as described in a previous monograph from this hospital (8).

## DATA OBSERVED

The "blood urea clearance," indicating the cubic centimeters of blood per minute cleared of urea by renal excretion, has been shown by Van Slyke, McIntosh, Möller, Hannon, and Johnston (7) to be a sensitive indicator of changes in renal function. The urea clearance tests were conducted as proposed by Möller, McIntosh and Van Slyke (2) with the patient at rest in bed. It is important to stress the latter point because Van Slyke, Alving and Rose (6) have shown that the clearance in some of the more advanced cases may be significantly reduced by allowing the patient to walk about during the two-hour period of the test. We have recorded the results in percentages of the average normal clearance.

Phenolsulphonphthalein excretion tests were also conducted at the same time as the urea clearance.

Under "duration of disease" the time is given from apparent onset until operation was performed.

Edema, blood pressure, and eye ground examination were usually recorded within a reasonably short period of operation. We have made no effort to call attention to the detail of the eye ground examination,

and where we record negative we mean that no gross change, such as hemorrhage and edema, is present

Hemoglobin is given in terms of oxygen capacity (20.7 volumes per cent being normal for adult men, 19.0 for women) (4)

The normal value for total plasma protein is about 7 per cent. When total protein falls below 5.2 to 5.8 per cent, or the albumin, normally averaging 4.3 per cent, falls below 2.3 to 2.7 per cent, edema is usually present (3)

The Addis sediment test gives the number of red blood cells and casts in a twelve hour specimen collected after a period of twelve hours in which the patient is on a dry diet (1). In many cases it has not been possible immediately after surgical operation to perform this test for obvious reasons. Protein in the urine is recorded for 24 hours, as determined by the sedimentation method of Shevky and Stafford (5)

*Histories of three cases in which renal function diminished after operation*

In the series of 31 cases there were 3 in which a marked fall in blood urea clearance followed operation. The histories of these 3 cases are given below. In one of them (No. 1), already in the terminal stage of renal disease and rapidly progressing, the urea clearance fell permanently after operation, which apparently accelerated the fatal course of the disease. In the other two patients the diminution of renal function after operation was temporary, and it does not appear that the progress of the disease was accelerated.

The first patient (No. 1) discovered that he had albuminuria and hematuria during the course of an insurance examination in June 1928. On his admission December 1928, he was edematous, had lost weight, and the blood pressure measured 166/86. Large quantities of albumin, many casts, and red blood cells were found in the urine. The course in the hospital was progressively down hill, terminating in convulsions and death. Features of interest during the course of the disease were, venous thrombosis in the popliteal fossa (December 19), marked increase in the formed elements in the urine following slight upper respiratory infection (March 15), gross hematuria resultant from a streptococcal pharyngitis (April 19), and right bundle branch block on June 9. The urea clearance on admission was 27 per cent of normal and fell progressively to 16 per cent on May 8. On May 11, tonsillectomy and adenoidectomy were performed under gas-oxygen-ether. No gross hematuria was noted after the operation. On May 23, the patient had still not recovered from the operation. He vomited frequently, and was weak, drowsy, and dehydrated. Complete suppression of urine occurred from 6 p.m. on May 23, until he was catheterized at 8 p.m. on May 26. The catheterized specimen contained no acetone or acetoacetic acid, and the specific gravity was 1.017. The plasma pH was 7.31, CO<sub>2</sub> content 20.58 mM per liter and chloride 72.1 mM per liter on May 27. It was not until June 16, that the patient was again bright and cheerful. At that time it was found that the clearance had fallen to 2.4 per cent of normal. Death occurred in uremia on July 2nd.





TABLE I (continued)

Case number and clinical data	Date	Blood					Urine (excretion per 12 hours)				Renal function	
		Urea nitrogen	Hemoglobin	Plasma proteins			Protein	Formed elements			Phthalein excretion (2 hours)	Urea clearance
				Albu- min	Glob- ulin	Total		Erythro- cytes	Leucocytes and epithe- lial cells	Casts		
No 2 (6238), male 16 years Terminal, hemorrhagic nephritis, duration ten weeks Blood pressure 148/87 (February 15, 1928), 162/110 (August 16, 1929), 150/110 (De- cember 17, 1929), eye grounds normal, no ede- ma	1928 February 15	mgm per 100 cc	volumes per cent O <sub>2</sub>	per cent	per cent	per cent	grams	millions	millions	thousands	per cent	per cent of normal
	March 6	55	12 5		3 0	2 8		94 0	100 1	6742	14 8	13 2
	March 7	Tonsillectomy—rapid loss of weight and dehydration for one week after operation										
	March 9	48		2 7	2 7	5 5		129 5	192 5	7700	11 8	15 0
	March 16	88	10 6	2 8	3 5	6 4		32 0	43 2	1800	6 7	9 7
	April 2	35		2 4	3 2	5 7		41 0	93 6	1600	10 2	20 4
										some renal failure casts		
	June 7	48	11 9	3 1	2 3	5 4		60 8	46 4	3040	16 0	17 5





TABLE I (continued)

No 3 (continued)												
1930	January 27	5	16 8	20	2 5	4 6	1 1	1 3		320	81	95
	February 3	20									58	21
	March 10	5									73	94
	August 26	33									18	13
1931	June 1	19									36	44
	August 4	17		2 5	30	5.5	80	0 5		4050	58	47
	August 20	Tonsillectomy and adenoidectomy										
	August 25	18		20	3 4	5.5	6 1	0 5		1960	48	50
	September 8	14									46	45
	September 27	15					3 8	0 1		620	54	58

TABLE II  
Cases without obvious change in renal function after operation

Case number and clinical data	Date	Blood					Urine (excretion per 12 hours)				Renal function	
		Urea nitrogen	Hemoglobin	Plasma proteins			Protein	Formed elements			Phthalein excretion (2 hours)	Urea clearance
				Albumin	Globulin	Total		Erythrocytes	Leucocytes and epithelial cells	Casts		
No 4 (6538), female 25 years Latent, hemorrhagic nephritis for 8 months Blood pressure 130/90, eye grounds negative, edema ++	1928 October 23	mgm per 100 cc	volumes per cent O <sub>2</sub>	per cent	per cent	per cent	grams	millions	millions	thousands	per cent	per cent of normal
	October 23	17	18.2	2.1	3.3	5.4		1.9	3.2	125		55
	November 5	2 teeth roots extracted										
	November 13	9						0.3	1.5	48		90
	December 4	9	18.0	2.2	1.9	4.1		0.03	0.6	18	65	87
	December 15	2 teeth extracted										

TABLE II (continued)

No 4 (continued)													
1929	9	11	15 9	2 6	2 5	5 1	2 7	1 1	2 2	259	65	92	
January	24	18	17 1				8.3	0 1	3 1	115	52	96	
February	9	16		2 5	2 2	4 7					59	68	
March	5	8	14 9	2 8	2 7	5 6	1.3	0.3	4.3	60	54	121	
March	14	7	17 1	2 7	2 7	5 5					65	161	
March	27	13	17 9	2 7	2 7	5 5					65	80	
April	6	molar extracted											
April	9	9	17 6	2 7	3 2	5 9					60	118	
May	1	9	18 5	3 4	2.3	5 7					60	52	
May	14	12	18 1	3 4	2.5	6 0	1.2	2 8	5 9	71	63	45	

TABLE II (continued)

Case number and clinical data	Date	Blood				Urine (excretion per 12 hours)				Renal function	
		Urea nitro- gen	Hemo- globin	Plasma proteins		Protein	Formed elements			Phthalein excretion (2 hours)	Urea clear- ance
				Albu- min	Glob- ulin		Total	Erythro- cytes	Leucocytes and epithe- lial cells		
No 5 (5681), male 8 years Active, chronic hemor- rhagic nephritis for 4 months Blood pressure 126/70, eye grounds neg- ative, edema ++	1926 May 3	mgm per 100 cc	volumes per cent O <sub>2</sub>	per cent	per cent	grams	millions	millions	thousands	per cent	per cent of normal
	May 6	36	14 2	2 1	4 2	3 6	+++++	+++++	++++	53	30
	May 17	25				4 0	+++++	+++++	++++	15	11
	May 21	tonsillectomy									
	May 28	29				5 0	+++++	++	++	10	20
	June 3	30	8 2			6 0	+++++	++	++	8	20
	June 22	26	9 1	2 4	1 8	4 0	gross			8	20
	July 8	28				2 8	+++++	++	++	10	19
	July 16	29				1 0	+++++	+++++	++	8	30
	July 29	20				3 0	+++++	++	++	29	32
	August 16	19	11 0			2 5	+++++	++	+		40
	August 27	17	13 2	2 7	5 2	4 2	+++++	++	+	38	43
	September 10	19	10 8			3 0	+++++	++	+	62	41
	September 20	14	13 8			2 1	+++++	++	+	62	70







TABLE II (continued)

No 8 (7388), male, 24 years Active and latent hemorrhagic nephritis for 15 months. Blood pressure 152/88, eye grounds small old white plaque near disc, edema +	1930	12	18.3	3 0	2.2	5 2	0 7	3 1	4 2	1 9	6 7	6 5
	April 21	12	18.3	3 0	2.2	5 2	0 7	3 1	4 2	1 9	6 7	6 5
	May 3	17					1 1	1 4	3 4	5 8	6 6	5 7
	May 3	abscessed tooth removed										
	May 5	16									7 2	5 5
	May 19	16									7 1	3 3
	June 9	7					1 2	2 0	1 6	1 1 5	7 1	8 0
	June 18	6									7 0	7 8
	June 19	tonsillectomy and adenoidectomy										
	June 28	20						No reaction in urine			6 1	2 5
	June 30	9									7 9	8 4
	July 29						1 2	0 6	1 7	1 3 6		
	September 30	12					2 0	0.3	0 6	3 2	5 0	7 7
								No hematoma has been found since on two yearly visits				

In this case in spite of difficulty of interpretation due to the rapidly progressive and virulent qualities of the disease it would appear that operation speeded the course

The second patient (No 2) suffered from hemorrhagic Bright's disease in the terminal stage. The disease was progressive and caused a rapid loss of kidney function. The patient was continually beset with complications such as abscesses, sties, conjunctivitis, edema and tonsillitis. His temperature was slightly elevated, his appetite poor, and he had vomited a number of times on previous days. In spite of the adverse condition of the patient, the tonsils were considered so badly infected that their removal was indicated. Following operation his temperature rose to 101.6°, nausea persisted. The ninth day after operation the clearance had fallen to 10 per cent of normal. The patient recovered satisfactorily from the operation but directly following the tonsillectomy the patient seemed to be on the verge of uremia.

The third patient (No 3) had Bright's disease with a marked nephrotic tendency. On November 17, one tooth was extracted which proved to be normal. There was no fever directly following the operation, although there had been fever for the three previous days. However, for the first time since admission red blood cells in pathological quantities were found in the urine sediment. On November 19, the tooth socket seemed in good condition but the tonsils were swollen and pharyngitis was present. There was a moderate decrease in the urine output, but this decrease had begun three days before the extraction. A marked fall in the urea clearance occurred. This would be a rather convincing indication of the effect of the operation on the ability of the kidney to excrete urea had not a very similar episode occurred a month later (January 6 to January 15) when no operative procedure was employed. Oliguria, hematuria and general prostration were evident. After recovery from this incident the clearance rose sharply to 95 per cent on January 27, but fell again to 21 per cent on February 3. From this period his clearance fluctuated markedly and usually without any apparent reason. He seemed peculiarly susceptible to upper respiratory infections. On August 20, of the following year, tonsillectomy and adenoidectomy were performed without effect on the kidney function and the excretion of red blood cells. The patient recovered rapidly from the operation.

#### DISCUSSION

Except for the three cases which are discussed in detail above, the operations on 31 nephritic patients caused no significant fall or rise in the kidney function as measured either by the urea clearance or phenolsulphonphthalein excretion.

Of the 28 cases who showed no significant renal effects after operation, it has not seemed necessary to report complete data. The observations in Table II covering five of these cases, suffice to show the nature of the results.

The plasma proteins do not appear changed to any evident degree by operation, whereas hemoglobin shows a tendency to fall slightly. Whether the latter is due to the loss of blood at the operation, or is to be credited to the natural progress of the disease is not certain. Urinary protein is not significantly altered. In some cases an increase in hematuria has been noted following operation, in some no change, and in still

others a reduction is found. Casts are equally erratic in their appearance. In all respects the effects appear to have been transient, and to have disappeared from a few days to a fortnight after the operation.

The data on the 31 cases discussed above do not cover periods sufficiently long after operation to bear on the effect of the operations on the ultimate course of the disease. However, the charts published in a previous monograph from this hospital (8) do appear to provide some basis for an opinion on this question. Of the 50 cases of acute and chronic hemorrhagic renal disease there reported, seventeen (Numbers 1, 3, 4, 7, 10, 12, 13, 17, 18, 25, 26, 27, 29, 31, 33, 36, 48) were subjected to operation, usually tonsillectomy, and were observed for months or years thereafter. Comparison of these charts with those of similar cases that were not subject to operation gives, we believe, a definite impression that the tonsillectomies were without effect, either favorable or unfavorable, on the course of the disease. The natural variability of the disease makes it impossible to state dogmatically this conclusion. We believe, however, that anyone who will compare the series of charts referred to with charts of unoperated cases in the same monograph will have difficulty in finding evidence for any effect of the operation on the course of the renal disease.

Experience with these and other cases has led to the conclusion in this clinic that in nephritic patients removal of tonsils, adenoids, and teeth are operations to be undertaken or not from the standpoint of the general hygiene of the patient, and without expectation that his renal disease will be benefited. On the other hand, unless the general condition is bad, it does not seem necessary to refuse an operation because of a somewhat diminished renal function. If the disease has been very active and the general condition poor, there may be danger of bringing on uremia in the days following the operation. But such danger seems to be remote, in subjects with blood urea clearances above 20 per cent of normal, if the general condition is not depressed by factors other than renal retention.

#### CONCLUSIONS

The operations of tonsillectomy, adenoidectomy and tooth extraction have usually had no effect on kidney function as measured by the urea clearance or the phthalein excretion. In 3 out of 31 cases, however, renal function was depressed in the days immediately after operation, and in one case, already terminal, uremic symptoms were brought on.

2. Red blood cells and casts in the urine were frequently increased for some days after operation. Such increase usually occurred without any fall in renal function.

3. Plasma protein content and urinary protein excretion were not affected by these operations. The hemoglobin content of the blood was in some cases somewhat decreased, perhaps as the result of loss of blood during the operation.

4 The results, together with more prolonged observations presented in a previous report (8), lead to the conclusions that removal of tonsils, adenoids, or teeth usually has no effect, favorable or unfavorable, on the progress of renal disease, but that when the general hygiene of a renal patient demands such an operation it is not necessary to refuse it, unless the renal disease is in the terminal stage (urea clearance consistently below 20 per cent of normal), or the general condition of the patient is unfavorable

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## STUDIES OF UREA EXCRETION VII

### THE EFFECTS OF POSTURE AND EXERCISE ON UREA EXCRETION

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The studies reported in this paper were undertaken primarily to ascertain whether, in determining the blood urea clearance<sup>1</sup> as a measure of renal function, the previously utilized precaution of keeping the subject at rest in a recumbent position is necessary, and whether the mild exercise and change of posture associated with walking about has sufficient effect on renal activity to alter the clearance values obtained. Observations in the two conditions have been made on three normal men and nineteen nephritic subjects with varying grades of renal impairment. The effect of change of posture from lying in bed to sitting in a chair has also been studied. Furthermore, in three subjects with normal renal function, the effect was studied of exercise of the maximum severity which could be maintained for the 2-hour period.

There is evidence that renal function in many details is affected by mere change in posture. Edel (5) in 1901 observed that change from the recumbent to the standing position decreased markedly the volume output of urine, and in subjects with cyclic albuminuria increased the protein excretion. Subsequent authors (6, 9, 15, 17, 22) have confirmed this observation, and have found that the excretion rate, not only of water, but of most of the other normal urinary constituents, is diminished by the change from recumbent to standing posture. Thus White, Rosen, Fischer and Wood (22) found that the average effect in three normal men of changing from lying to standing posture was to diminish the hourly excretions to the following proportions of the outputs in the recumbent position: urine volume to 30 per cent, chlorides to 45 per cent, phosphorus to 67 per cent, sulfur to 71 per cent, urea to 61 per cent, and creatinine to 91 per cent. Cordero and Friedman (4) found that change from recumbent to upright position caused an average decrease of 10 to 13 per cent in the proportion of injected phenolsulfonephthalein excreted in 2 hours by normal subjects.

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<sup>1</sup>The terms, "standard blood urea clearance" and "maximum blood urea clearance" used in expressing the urea excreting efficiency of the kidneys have been defined in the second paper of this series (12).

As possible causes of such effects, Erlanger and Hooker (6) and White, Rosen, Fischer, and Wood (22) observed that the change from recumbent to upright position caused in 30 to 60 minutes a concentration of the blood by seepage of 10 to 11 per cent of the total plasma fluid into the leg tissues, and also that there was a decrease of 10 to 15 millimeters in pulse pressure. That the urine flow is diminished because increased osmotic pressure of the more concentrated plasma proteins retards glomerular filtration and favors tubular reabsorption of water, was believed by Ni and Rehberg (15), while Erlanger and Hooker (6) were inclined to see the chief retarding factor in the decreased pulse pressure and the number of functioning glomeruli.

Whether the urea clearance is affected depends upon whether the relationship between the outputs of urea and water is that allowed for in calculating the clearance. When the volume is below the augmentation limit of about 2 cc per minute, the calculation of the clearance allows for change of urea excretion rate in proportion to the square root of the urine volume, while with higher volumes urea excretion rate, estimated as the "maximum clearance" (11), is assumed to be independent of urine volume. If standing up does not retard the urea output more than accords with the above allowances for urine volume effect, the value of the clearance will not be affected.

In regard to this question the only data available appear to be observations made by Addis and Drury (1) and MacKay (10) on the effects of vigorous exercise. Addis and Drury found that in a normal subject the maximum blood urea clearance during an hour of continuous running was decreased to 70 per cent of the value observed during rest. MacKay found that during a 2-hour tennis match the standard clearance of a normal man was lowered to about half its usual resting value.

In previous studies by Moller, McIntosh, and Van Slyke (12, 13) administration of 15 grams of urea before the observation period was found to have no significant influence on the clearances of either normal or nephritic subjects resting in bed. Professor G. A. Harrison of St. Bartholomew's Hospital in London has personally communicated to the writers some observations of blood urea clearances during successive hourly periods in several subjects who were up and about. In some cases 15 grams of urea were given before the day's series of observations, while on other days the same subjects were observed without urea administration. It appeared from Professor Harrison's preliminary observations that urea administration to subjects who were up and about might exert a stabilizing effect on their clearance. Professor Harrison did not wish to pursue the question farther. We have accordingly included in the present studies observations in which urea was given and withheld on alternate experimental days.

## METHODS

The *standard blood urea clearance*,  $C_s = \frac{U}{B} \sqrt{V_c}$ , (11, 12) was calculated in all instances in which the volume of urine, corrected for surface area (11) was below the augmentation limit of 2 cc per minute. When the corrected volume was above this limit the *maximum blood urea clearance*,  $\left( C_m = \frac{UV_c}{B} \right)$ , was used. In the above formulae,  $U$  represents the urine urea concentration,  $B$  the blood urea concentration, and  $V_c$  the urine volume in cubic centimeters per minute, corrected for body size as described by McIntosh, Möller, and Van Slyke (11). On the charts and in the tables the clearance has been recorded in percentages of the normal mean standard clearance of 54 cc per minute or of the normal mean maximum clearance of 75 cc. per minute.

The blood and urine urea concentrations were determined by the gasometric urease method (19).

All blood urea clearance tests were performed in the morning. Each patient received at 7 a.m. an ordinary breakfast, unrestricted except for coffee, which was uniformly omitted. Urine was voided at 8.30 a.m. and the specimen was discarded. The urines voided during the two 1-hour periods 8.30–9.30 and 9.30–10.30 were then collected separately. At about 9.30 blood was drawn for urea determination.

Whenever feasible, observations were carried out while the subjects were, on alternate days, in bed and walking about, or in bed and sitting up. Similar studies were made after urea ingestion, 15 grams of urea by mouth being given on the mornings of these tests.

One patient (No. 19), normal except for a slight albuminuria, exercised vigorously by walking up and down the stairs of the hospital on the day of the test. Two normal individuals exercised by playing squash-racquets during the 2 hours of the tests.

Clinical details other than the blood urea clearance will not be given, because they do not appear of significance to these conclusions. The subjects represented in Figure 1 were separable with regard to renal condition as follows. Nos. 1, 2, 3, 4, 5, 6, 7, and 10, were in the terminal stage of hemorrhagic Bright's disease, as defined in a previous paper (21). Nos. 9, 12, 16, 17 and 18 were in the active chronic stage of the same disease. Nos. 11, and 20 were degenerative, and 13 was sclerotic. Nos. 19, 21, 22, and 23 were normal young adults, except that 19 had an occasional slight albuminuria.

## RESULTS

The urea clearance values are shown in Figure 1. In Tables 1 and 2 the data in detail are given for the observations on one normal subject and on one nephritic, Subject 6. The latter case was selected for presentation in detail because the subject, more than any other in our series,



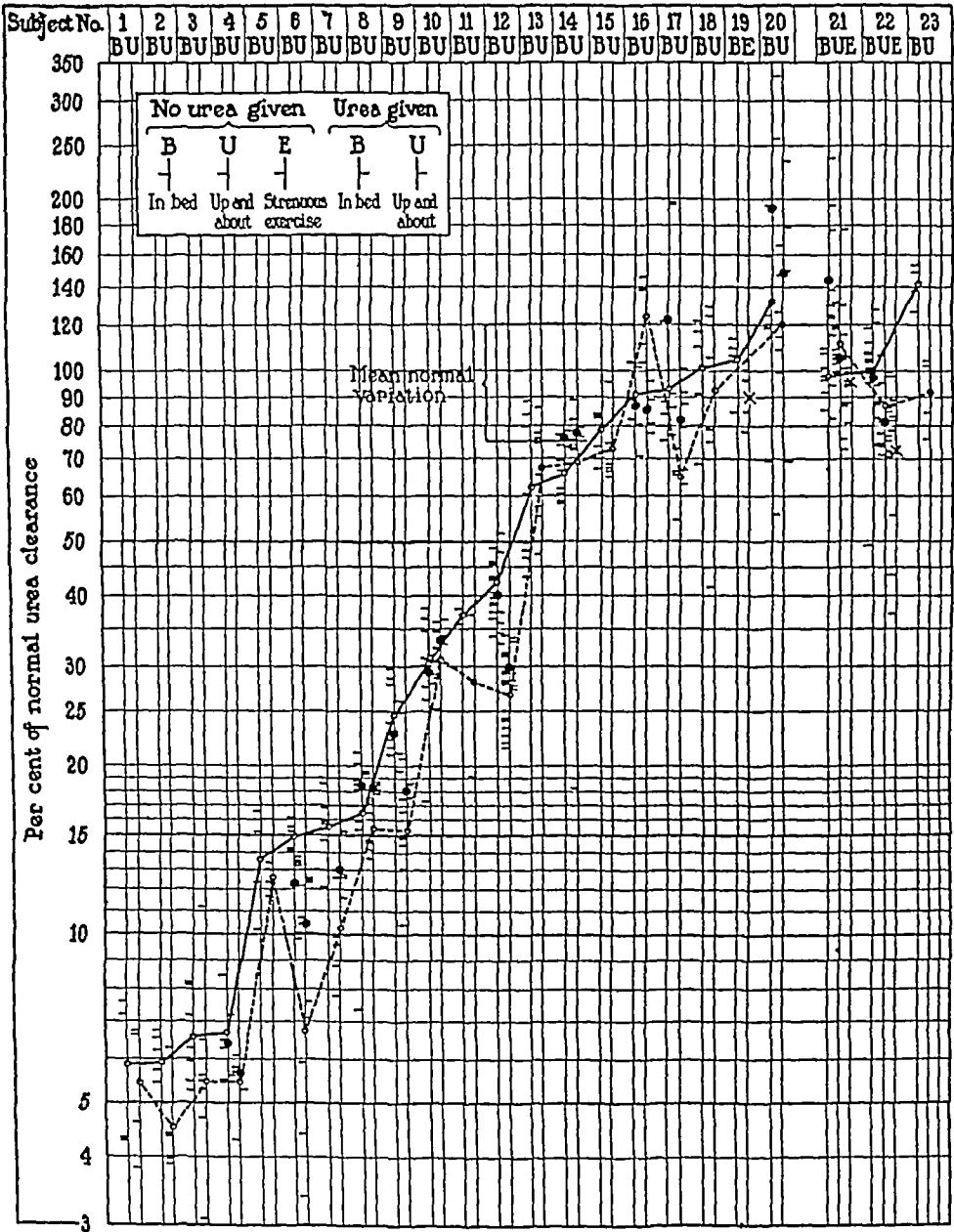


FIG 1

Solid line connects average clearance values taken while subjects were in bed. Dashed line connects average clearance values taken while subjects were up and about. Hollow circle indicates average of all clearances on a subject, solid circle indicates average of clearances taken after administration of urea. X mark indicates average of clearances taken during hard exercise. Short horizontal lines indicate individual clearance values.

TABLE 1

*Observations on A.II, terminal hemorrhagic nephritic showing maximal effect on urea excretion caused by change from bed rest to moderate activity*  
*Case No 6 Hospital No 7525*

Conditions of observation		Date	U <sub>excr</sub>	U	B	$\frac{U}{B}$	Blood urea clearance $\frac{U \cdot P}{B}$ or $\frac{UV}{B}$
		1930-31	cc. urine per minute	mgms urea N per 100 cc. urine	mgms urea N per 100 cc. blood	Concen- tration ratio	per cent of normal
No urea given	In bed	November 25	0.560	436	42.6	10.2	14.2
		"	1.12	350	42.6	8.2	16.1
		November 28	0.608	431	44.2	9.8	14.1
		"	0.832	407	44.2	9.2	15.5
		December 9	0.592	404	41.0	9.9	14.1
		"	0.896	357	41.0	8.7	15.3
		Average	0.77	398	42.6	9.3	14.9
	Up and about	November 26	0.144	202	42.1	4.8	3.4
		"	0.464	405	42.1	9.6	12.2
		November 29	0.256	274	43.5	6.3	5.9
		"	0.400	387	43.5	8.9	10.4
		December 10	0.126	272	41.0	6.6	4.4
		"	0.320	417	41.0	10.2	10.7
		Average	0.29	326	42.2	7.7	7.8
15 grams urea by mouth before each test	In bed	December 16	1.47	332	55.1	6.0	13.5
		"	1.12	359	55.1	6.5	12.8
		December 18	1.50	444	72.8	6.1	13.8
		"	1.95	381	72.8	5.2	13.5
		January 13	0.895	471	82.6	5.7	10.0
		"	0.943	451	82.6	5.5	9.8
		Average	1.31	406	70.2	5.8	12.2
	Up and about	December 17	0.416	534	63.2	5.5	10.1
		"	0.704	507	63.2	6.7	12.5
		December 19	0.880	526	72.2	6.8	12.6
		"	0.768	556	72.2	6.8	12.5
		January 14	0.480	545	92.1	4.1	7.6
		"	0.416	546	92.1	3.8	7.1
		Average	0.611	536	75.8	7.2	10.4

shows the depressing effect on urea excretion as the result of changing from bed rest to walking about

*The effect of mild physical activity on the blood urea clearance*

*In normal subjects, and in nephritic patients with relatively slight functional impairment (over 50 per cent of normal clearance) (Subjects 13 to*

23), being up and about caused no significant change in the urea clearance from the values observed when the subjects were in bed before and during the excretory periods. In some cases the average clearance obtained when the subjects were up and about was lower, in others higher, than when in bed. As shown by the data of Table 2, given in full for a normal

TABLE 2  
*Observations on A A, Normal subject, No 22*

Conditions of observation		Date	$V_{cor}$	$U$	$B$	$\frac{U}{B}$	Blood urea clearance $\frac{U\sqrt{V}}{B}$ or $\frac{UV}{B}$
		1931	cc urine per minute	mgms urea N per 100 cc. urine	mgms urea N per 100 cc blood	Concentration ratio	per cent of normal
No urea given	In bed	January 6	2 61	533	15 6		119
		"	4 09	306	15 6		107
		January 13	1 23	871	18 3	48	98
		"	2 30	289	18 3		(48)*
		January 23	1 22	879	16 7	52	107
		"	1 53	722	16 7	43	99
		February 10	1 52	572	13 7	42	95
		"	2 72	425	13 7		112
		February 12	1 58	747	16 7	45	104
		"	1 85	687	16 7	41	104
		Average	2 07	603	16 2	45	105
	Up and about	January 7	0 979	752	15 8	48	87
		"	1 03	681	15 8	43	81
		January 14	0 710	1148	18 9	61	95
		"	0 896	1148	18 9	61	106
		January 22	1 23	837	22 3	38	77
		"	1 20	775	22 3	35	71
		January 30	0 957	853	15 7	54	106
		"	0 833	761	15 7	49	100
		February 3	0 458	964	16 5	59	74
		"	0 537	896	16 5	55	74
		Average	0 880	882	17 8	50	84
	Vigorous exercise	January 8	0 576	950	16 3	58	82
		"	0 945	878	16 3	54	97
		January 15	0 803	920	19 6	47	78
		"	0 831	957	19 6	49	83
		January 31	0 437	1101	15 5	71	87
		"	0 453	1098	15 5	71	88
		March 28	0 265	1044	18 1	58	56
		"	0 365	1155	18 1	64	71
		March 29	0 349	557	16 4	34	37
		"	0 409	603	16 4	37	44
		Average	0 544	926	17 2	54	72

TABLE 1 (continued)

No.	Patient	Diet	Time	V <sub>u</sub>	S	C	C <sub>u</sub>	C <sub>u</sub> /C
				ml/min	mg/100 ml	mg/100 ml	mg/100 ml	
13	Patient	Fast	12:00-1:00	1	1.0	1.0	1.0	1.0
			1:00-2:00	1.4	1.1	1.1	1.1	1.1
			2:00-3:00	1.5	1.1	1.1	1.1	1.1
			3:00-4:00	2.5	1.1	1.1	1.1	1.1
			4:00-5:00	1.1	1.1	1.1	1.1	1.1
			5:00-6:00	1.1	1.1	1.1	1.1	1.1
			6:00-7:00	1.1	1.1	1.1	1.1	1.1
			7:00-8:00	1.1	1.1	1.1	1.1	1.1
			8:00-9:00	1.1	1.1	1.1	1.1	1.1
			Average	1.1	1.1	1.1	1.1	1.1
14	Patient	Fast	12:00-1:00	1.1	1.1	1.1	1.1	1.1
			1:00-2:00	1.1	1.1	1.1	1.1	1.1
			2:00-3:00	1.1	1.1	1.1	1.1	1.1
			3:00-4:00	1.1	1.1	1.1	1.1	1.1
			4:00-5:00	1.1	1.1	1.1	1.1	1.1
			5:00-6:00	1.1	1.1	1.1	1.1	1.1
			6:00-7:00	1.1	1.1	1.1	1.1	1.1
			7:00-8:00	1.1	1.1	1.1	1.1	1.1
			8:00-9:00	1.1	1.1	1.1	1.1	1.1
			Average	1.1	1.1	1.1	1.1	1.1

\*On 50 per cent average

while the rather marked renal effect of the change in posture and activity in the urine volume, much less being exerted while the subject is up and about than when in bed. This phenomenon was uniformly noted both in the subjects with partial renal function and in those with diminished function. The urea concentration in the smaller volume of urine was increased only slightly. However, to prevent the clearance figures from varying except in one or two isolated observations, markedly outside the range of clearances observed during bed rest in this group of subjects (Nos. 11 to 23).

In representative subjects with less than 50 per cent of normal clearance (Subjects 1 to 12 inclusive) being up and about uniformly depressed the average clearance below that observed during bed rest, except in one patient (No. 10) where there was no change. In three fourths of the cases the effect was relatively slight and not sufficient to influence the diagnostic import of the clearances, but in cases 6, 9, and 12 being up and about lowered the mean clearance to 60 per cent or less of that observed during bed rest. It is evident therefore that in nephritics with marked renal impairment clearance determinations should continue to be made under standard conditions of rest.

During approximately half of another series of clearance tests each subject sat in a chair. The other clearances of the series were taken with the subjects in bed. The results are shown in Figure 2. They indicate no demonstrable effect of this change of posture on the clearance.

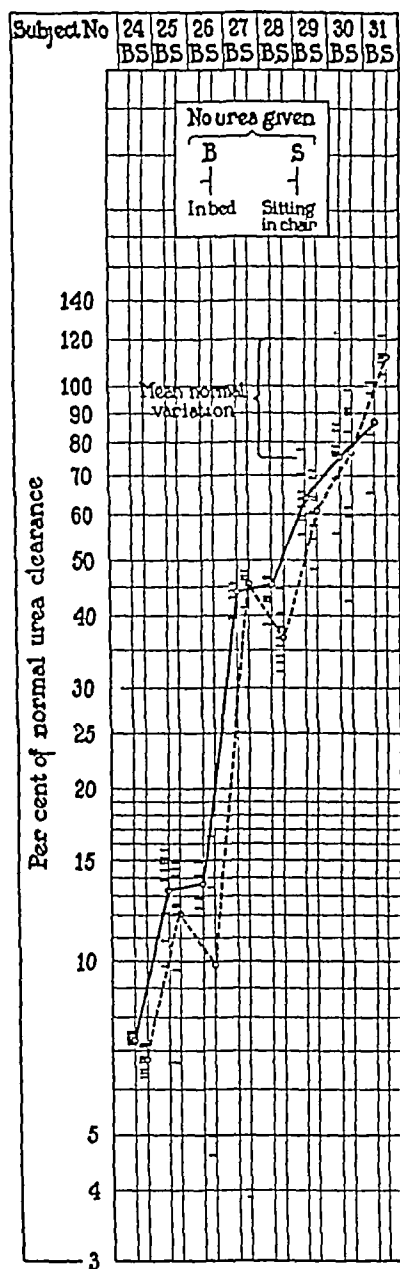


FIG 2

Solid line connects average clearance values taken while subjects were in bed. Dashed line connects average clearance values taken while subjects were sitting. Short horizontal lines indicate individual clearance values.



compared together, while the two sets taken after urea administration form another group comparable among themselves (because of approximate constancy of the blood urea content) Moller, McIntosh, and Van Slyke (12) found a similar behavior in nephritics of all grades of severity when the subjects were tested during rest in bed. The concentrations of urea in the urine compared with the concentrations in the blood were much smaller in advanced nephritis than in normal subjects, but the variation with urine volume in a given subject was of the same nature.

In nephritic subjects Nos. 6, 9, 12, however, who show marked fall in blood urea clearance caused by leaving bed and walking about, this inverse relationship between urine volume and concentration fails to hold. In No. 6 (Table 1), who shows, of the subjects reported in this paper, the most depression of clearance caused by leaving bed and walking about, there is in the first two sets of data, taken without urea administration, no inverse relationship at all. The urine volume, in the observations made when this subject was up and about averages only 40 per cent as great as the volume when he was in bed, but the urea concentration in the smaller volume, when the patient was up, averages actually somewhat less than in the larger volumes excreted in bed. It is this failure, partial or complete, to increase normally the urea concentration in the smaller volumes of urine passed while up and about, that causes the clearance to be markedly depressed by the latter condition in three of the nineteen nephritics studied. The depression, although evident only in nephritic cases with less than 50 per cent of normal clearance, was not at all directly related to the severity of renal impairment. In some of the most advanced cases being up and about depressed the clearance but little.

#### SUMMARY

In normal subjects, or in nephritics with more than 50 per cent of normal renal function measured by the blood urea clearance, the clearance has not been found to be essentially different, whether the subjects were kept in bed during the 2-hour observation periods or were walking about. Urine volume was uniformly less while the subjects were up and about, but the effect in depressing urea excretion was about that allowed for in the formulae for calculating the standard and maximum clearances, so that the values of the latter were not markedly affected.

In three of the twelve nephritics observed with less than 50 per cent of normal renal function, measured by the blood urea clearance observed with the subjects in bed, rising and walking about depressed the clearance markedly, the mean values observed being 44, 60, and 67 per cent respectively of those observed in the same patients during bed rest.

Severe exercise taken by three subjects with normal renal function depressed the clearance somewhat, as found by Addis and Drury (1) and MacKay (10), but in only three out of twenty-two clearances determined

during heavy exertion were the values definitely abnormal, i e, below 70 per cent of the mean normal level

Administration of 15 grams of urea before the observation periods did not significantly affect the results

It appears that in normal subjects and in nephritics with more than 50 per cent of average normal clearance, the clearance values can be determined without loss of accuracy while the subjects are up and about. In nephritic patients with less than 50 per cent of normal clearance values, however, it is essential to keep the subject at rest in a recumbent position during the 2 hour excretion period, if the results are to be compared with those heretofore obtained by the clearance test

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STUDIES ON THE COURSE OF VASOMOTOR FIBERS AS  
MEASURED BY THERMIC CHANGES IN THE FEET  
AFTER ARTERIAL LIGATION AND SECTION OF  
THE SPINAL CORD AT VARIOUS LEVELS

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ERRATA

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Articles by Alfred E Cohn, J Murray Steele and Harold J Stewart—  
Studies on the Effect of the Action of Digitalis on the Output  
of Blood from the Heart I to III Pages 871 to 955

Page 874, line 5, for "of" read "on"

Page 892, footnote 4, line 2, for "them" read "these"

Page 922, Figure 2, Digitan 1 0, for "mg" read "gm"

Page 924, Figure 4, Digitan 1 0, for "mg" read "gm"

Page 928, Figure 7, line 2 of legend title, for "1" read "2"

Page 938, Table 3, lines 2 and 4 from the bottom, read "1 3" and "dis-  
continued" under Digitan

Page 947, Figure 15, for Digitan "1 0 on Jan 6" read "0 1 on Jan 9"

6) have stated that in an animal with seven lumbar vertebrae the con-  
nector fibers to the sympathetic ganglia supplying the posterior extremity  
are contained in the 11th, 12th and 13th thoracic and the 1st, 2nd and 3rd  
lumbar spinal roots Since the results of the above (3) experiments did  
not coincide with their findings it seemed desirable to conduct further  
experiments to determine the effect of transection of the spinal cord at  
various levels

METHOD

As in the previous experiments, dogs were given sodium amytal (Lilly)  
intraperitoneally and the temperatures of the room, rectum and hind feet

recorded simultaneously by means of thermocouples attached to a Leeds and Northrup resistance thermometer. The experimental procedure was, however, modified in some respects. Unpublished experiments show that there is a correlation between body temperature and the temperature reaction of the extremities. The body temperature was therefore maintained at normal by use of blankets and by means of electric heating pads when necessary. Inasmuch as many of the experiments extended over a period of 24 hours, the preliminary intra-abdominal ligations of the arteries were accomplished using sterile technique to minimize general temperature reactions. During the course of the experiments it was found that transection of the spinal cord at a given level did not always result in the same changes in temperature in the extremity but that the effects varied considerably in the different animals, apparently under the same conditions. This was most frequently found when the level of transection was below the first lumbar nerve root.

Other investigators (Langley, (4) Gaskell (2), Bayliss and Bradford (1)) have demonstrated that vasomotor fibers supplying the lower limbs leave the spinal cord from the eleventh thoracic to the third lumbar nerves. It was therefore apparent that transection of the spinal cord at the lower levels would interrupt fewer vasomotor fibers than at the higher. It would seem that failure of the temperature to rise or of the blood flow to increase did not necessarily mean that no vasomotor fibers had been interrupted but rather that a sufficient number had not been cut to result in increased blood flow. It also seemed probable that if the femoral artery were ligated above the profunda branch, it would be necessary to interrupt more fibers in order to result in a rise of temperature of the foot than if the ligation were done below the profunda branch. Hence in some of these experiments the ligation was carried out in the groin below the profunda branch. The dogs were taken from stock without preliminary dietary standardization. Preference was given to young animals although some of them were obviously old.

Following the fall in temperature of the foot after ligation laminectomy was performed at the desired level, the dura was opened, and the spinal cord transected. When the cord was exposed the dentate ligaments at the desired level were cut and the cord gently lifted with a blunt hook and cut with scissors. There was usually very little bleeding which could readily be controlled by cotton pledgets or by placing a piece of muscle between the ends of the transected cord. Following this the temperature changes in the hind feet were noted as shown in the charts.

#### EXPERIMENTAL DATA

In this group of experiments in which the spinal cord was transected at various levels, twenty-one dogs were used. Two dogs failed to exhibit vasodilatation even after removal of the sympathetic chain and section of the sciatic nerve. One of these (Experiment 146, Protocols) was an old animal in poor condition, during amytal anesthesia this animal developed a marked bronchorrhea. One animal (Experiment 15) also failed to react both on transection of the cord and likewise following removal of the lumbar sympathetic chain. The femoral arteries of this animal were ligated at the bifurcation resulting in a slight fall only in the temperature of the feet. The temperature of the room was 80° F. Inasmuch as three hours following ligation, the temperature of the feet

was still 94° F it is probable that vasodilatation was already present and that this explains the absence of change following operation. Experiment 30 was also inconclusive. A fall in temperature following ligation of the arteries took place, but no rise occurred after transections at L4<sup>1</sup> and L2. Following transection of the cord at T10<sup>1</sup> an increase of 7° F occurred in the temperature of the feet, but was not sustained. At the end of two hours the feet were again at room temperature. Following this, removal of the sympathetic ganglia did not result in increase of the temperature of the feet.

All of the other eighteen dogs reacted well either to transection of the cord or removal of the sympathetic ganglia. The response obtained in each of the experiments will be found in the protocols. The terms "Immediate" and "Delayed" refer to the time when increase in temperature occurred and the terms "Abrupt" and "Gradual," to the type of curve. It is to be noted that vasodilatation always resulted when the cord was transected above the level of the second lumbar nerve root. However, in Experiment 30 there was none at L2, in Experiment 27 at L3, in Experiments 30 and 31 at L4 and in Experiment 139 at L5. In all of these experiments except 139 the ligation was at the bifurcation. In Experiment 139 the right side was ligated at the bifurcation and the left side below Poupart's ligament. When it was performed below Poupart's ligament (i.e. below the profunda branch) immediate vasodilatation took place at the lower levels, as in Experiments 135, 136 and 140.

#### DISCUSSION

The experiments reported in this paper seem to indicate that vasomotor pathways can be traced satisfactorily by this method. The results are, however, not always consistent as there are several variable factors which may lead to erroneous interpretation. It may be well therefore to review some of the variables in order that the results may be more easily interpreted.

The reaction of dogs to amytal anesthesia is not constant and its effect on the sympathetic nervous system is not well understood. These experiments demonstrate that some dogs under amytal anesthesia are no longer able to maintain normal temperature in a room of 70° F. Certain dogs do so, however, for long periods. While long haired dogs perform this function better than short haired dogs, this is not always the case. The important result is that dogs under amytal anesthesia conserve their body heat by vasoconstriction or vasodilatation, depending upon environmental temperature and the peculiarities of the individual dog. Vasoconstriction as a result of low body temperature may be sufficient to counteract the effect of interrupting a few vasoconstrictor

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<sup>1</sup>L4 represents fourth lumbar nerve root. T10 represents tenth thoracic nerve root. In each instance the transection was carried out immediately caudad to the root named.

fibers When the body temperature is normal, interruption of the same number of vasoconstrictor fibers is made manifest by a rise in the temperature of the limb in question In spite of the obvious effect of amyotal anesthesia on the conservation of body heat, relatively small changes in the reaction of the sympathetic nervous system may, nevertheless, be demonstrated providing body heat is artificially maintained at a normal level There is also considerable variation in the reaction of the vasomotor system of various dogs Certain animals under standard conditions respond with marked vasoconstriction while others apparently under the same conditions exhibit vasodilatation In addition to variation in physiological response there are also anatomical variations It was found that when the femoral artery was ligated above the profunda branch, the collateral circulation resulting from interruption of the sympathetic fibers was by way of the median sacral artery When the median sacral artery was ligated as well as the femoral, no rise of temperature could be produced in that limb Inasmuch as there is considerable variation in the portion of the limb supplied by these arteries, the experiments cannot be considered identical in the anatomical sense

Previous studies (Langley (4) (5) (6), Bayliss and Bradford (1), Gaskell (2)) have shown that vasomotor fibers leave the spinal cord from the level of the second thoracic to the third lumbar spinal nerve roots Various experimental methods were used in determining these levels In cats, Langley (5), observed the secretion of sweat in the foot pad as well as the color of the pad when the individual nerve roots were cut and stimulated Gaskell carried out extensive anatomical and histological studies on the nerve roots and rami communicantes of dogs which were essentially in agreement with the findings of Langley Bayliss and Bradford using a plethysmograph recorded the changes in volume of the limb following stimulation of the individual nerve roots and also agreed with the findings of Gaskell and Langley

The experiments recorded in the protocols demonstrate the quantitative aspects of vasodilatation depending on the number of fibers interrupted and the level of ligation as well as on the other factors enumerated above Transection of the spinal cord as low as the sixth lumbar nerve root resulted in vasodilatation of the vessels of the extremity providing the means for recording dilatation did not require the interruption of a large number of fibers In other words, a sufficient number of vasoconstrictor fibers leave the spinal cord below the level of the sixth lumbar nerve root to result in vasodilatation when these fibers are interrupted Transection of the spinal cord interrupts of course all of the fibers leaving the cord below this level It is probable that interruption or stimulation of a single nerve root containing a few fibers would not result therefore in sufficient vasodilatation to be recorded by the means used The response obtained by ligation at various levels indicates the

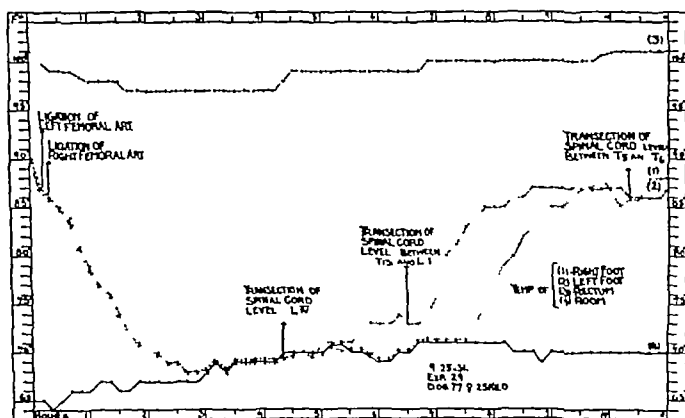


FIG I EXPERIMENT 29

The femoral arteries were ligated just below the bifurcation of the aorta. Curves show a slight rise of temperature of the left foot after transection of the spinal cord below L4 nerve roots. Transection below T13 resulted in an immediate, abrupt, sustained rise on the left, and a delayed, abrupt, sustained rise on the right.

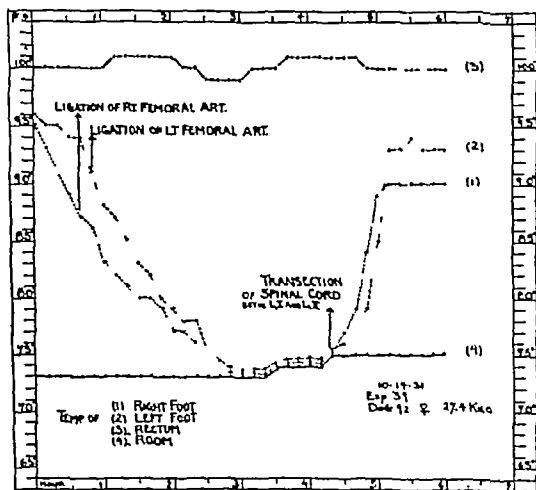


FIG II EXPERIMENT 39

The femoral arteries were ligated just below the bifurcation of the aorta. Transection of the spinal cord below L1 nerve roots resulted in an immediate abrupt sustained rise of the temperature of both feet.

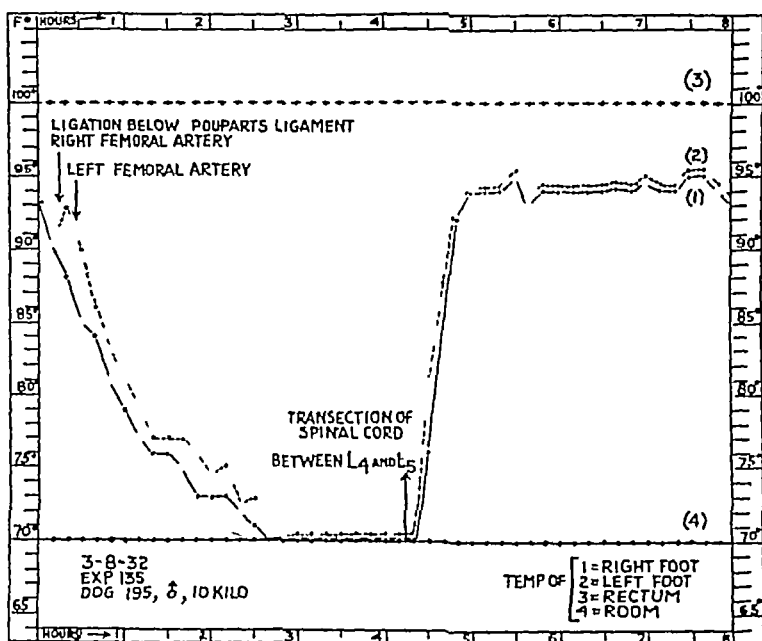


FIG III EXPERIMENT 135

The femoral arteries were ligated below Poupart's ligament (below profunda branch) Transection of the spinal cord below L4 nerve roots resulted in an immediate abrupt sustained rise of the temperature of both feet

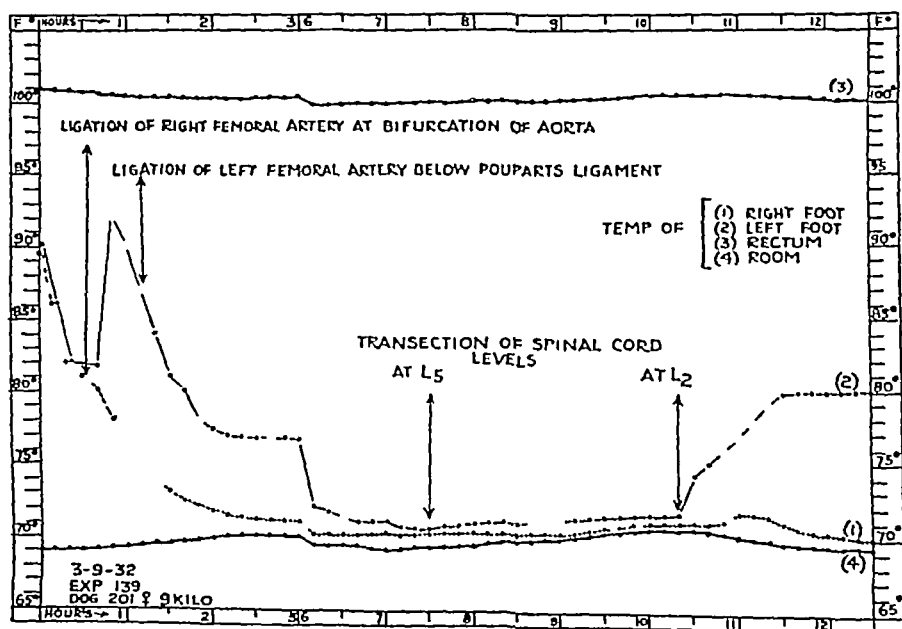


FIG IV EXPERIMENT 139

The curves show the difference in response to cord transection when the femoral artery is ligated just below the aorta on the right and below Poupart's ligament on the left

quantitative aspect of the methods used for recording vasodilatation, the limits of which have as yet not been fully determined. Further work on the course of vasomotor fibers is being carried on in this laboratory.

#### SUMMARY

1 The vasomotor effects of spinal cord transection at various levels have been studied in the dog following the ligation of the femoral artery at various levels.

2 The temperature changes in the hind feet following ligation of the arteries and transection of the spinal cord are dependent on both the level of transection of the cord (number of vasomotor fibers interrupted) and the level of ligation of the artery as well as on other factors which are discussed.

3 If the femoral artery is ligated just below the bifurcation of the aorta, transection of the spinal cord above the level at which the vasomotor fibers are given off to the hind limb is followed by immediate full vasodilatation. Under the same conditions transection of the spinal cord below the level of the first lumbar nerve root does not always bring about a vasodilatation of sufficient degree to be recorded by the method used in these experiments. This explains the failure to obtain vasodilatation as previously reported (3). Partial or incomplete vasodilatation may however, be observed in some animals.

4 If the femoral artery is ligated below the profunda branch (below Poupart's ligament) transection of the spinal cord at lower levels will cause immediate full vasodilatation.

5 Using this method it was found that there is a sufficient number of vasomotor fibers leaving the spinal cord below the level of the sixth lumbar nerve root to bring about immediate vasodilatation when they are interrupted.

#### CONCLUSIONS

These experiments suggest that, if the method of measuring vasodilatation is capable of recording the changes resulting from division of a small number of fibers, vasomotor fibers are found to leave the spinal cord at lower levels than have previously been described.

#### PROTOCOLS

##### *Summary of twenty one experiments in which the spinal cord was transected*

*Experiment 15* Room temperature 80° F femoral arteries ligated below bifurcation of the aorta spinal cord transected at level of T8. Result No response after transection of the cord no response after removal of the sympathetic ganglia. Vasodilatation present at time of transection, hence no further response.

*Experiment 16* Room temperature 80° F femoral arteries ligated below bifurcation of the aorta spinal cord transected at level of T5. Result There was an immediate abrupt sustained rise of 14° F in both feet.



*Experiment 19* Room temperature 82° F , femoral arteries ligated below bifurcation of the aorta, spinal cord transected at level of T5 Result There was an immediate abrupt sustained rise of 10° F in both feet

*Experiment 24* Room temperature 82° F , femoral arteries ligated below bifurcation of the aorta, spinal cord transected at level of L1 Result There was an immediate gradual sustained rise of 14° F in the right foot, and an immediate rise in the left foot from 94° to 100° F The temperature of the left foot had not decreased with that of the right

*Experiment 25* Room temperature 80° F , femoral arteries ligated below bifurcation of the aorta, spinal cord transected at level of T12 Result A slightly delayed but abrupt and sustained rise of 8° F in both feet

*Experiment 26* Room temperature 77° F , femoral arteries ligated below bifurcation of the aorta, spinal cord transected at level of L2 Result An immediate abrupt sustained rise of 12° F in both feet There was also a slight additional rise after sympathectomy

*Experiment 27* Room temperature 77° F , femoral arteries ligated below bifurcation of the aorta, spinal cord transected at the level of L3 Result There was no change in temperature after the transection but an immediate rise of 20° F after sympathectomy

*Experiment 28* Room temperature 72° F , femoral arteries ligated below bifurcation of the aorta, spinal cord transected at the level of L2 Result An immediate gradual sustained rise of 20° F in the right foot, a delayed gradual slight rise in the left foot, and a rise of only 6° F in the left foot after sympathectomy Following ligation, the temperature of the left foot fell much more abruptly to room temperature

*Experiment 29* Room temperature 70° F , femoral arteries ligated below bifurcation of the aorta With transection of the spinal cord at L4 there was an immediate gradual rise of 4° F in the left foot only, with transection at L2 there was an immediate abrupt sustained rise of 14° F in the left foot and a delayed gradual sustained rise of 16° F in the right foot With transection at T6 there was an immediate rise of 2° F in both feet

*Experiment 30* Room temperature 70° F , femoral arteries ligated below bifurcation of the aorta Transecting the spinal cord at the level of L4 elicited no response in either foot, as was also the case with transection at L2 Upon transecting the cord at the level of T10 there was an immediate gradual rise of 7° F in both feet lasting 2 hours, but again on transecting at the level of T5 there was no response There was no response after removal of the sympathetic ganglia

*Experiment 31* Room temperature 70° F , femoral arteries ligated below bifurcation of the aorta Transection of the spinal cord at the level of L4 gave no response, upon transection at the level of L2 there was an immediate gradual sustained rise of 10° F in the left foot and 3° F in the right foot, transection at the level of T10 was followed by an immediate abrupt further rise of 16° F in the left foot and 4° F in the right Transection at the level of T7 gave no response The temperature of the right foot had been 8° F below that of the left before ligation and the fall of the temperature of the right foot to room temperature after ligation of the femoral arteries was much more abrupt

*Experiment 39* Room temperature 73° F , femoral arteries ligated below bifurcation of the aorta The spinal cord was transected at the level of L1 There was an immediate abrupt sustained rise of 21° F in the left foot and 16° F in the right foot Upon transection of the cord at the level of L1 on the left the root was cut, but on the right the root was intact

*Experiment 41* Room temperature 74° F femoral arteries ligated below bifurcation of the aorta spinal cord transected at the level of L1 Result Immediate abrupt sustained rise of 22° F in both feet The rectal temperature of this animal was 103° F at the termination of the experiment.

*Experiment 71* Room temperature 72° F femoral arteries ligated below Poupart's ligament and below profunda artery spinal cord transected hemilaterally at the level of T9 on the left and at T10 on the right. Result Immediate abrupt sustained hemilateral rise of 18° F in the left foot, and a delayed abrupt sustained hemilateral rise of 18° F in the right foot.

*Experiment 72* Room temperature 70° F femoral arteries ligated below Poupart's ligament and below profunda artery An anterior quadrilateral section was done at the level of T10 on the left followed by a similar procedure on the right. Result There was an immediate abrupt sustained rise of 18° F in the left foot and a delayed gradual sustained rise of 4° F in the right foot A deeper anterior quadrilateral section was then carried out at the level of T12 on the right which was followed by an immediate abrupt further rise of 10° F in the right foot.

*Experiment 135* Room temperature 72° F femoral arteries ligated below Poupart's ligament and below profunda artery, spinal cord transected at the level of L5 Result Immediate abrupt sustained rise of 22° F in both feet.

*Experiment 136* Room temperature 70° F femoral arteries ligated below Poupart's ligament on both sides, and the profunda artery was also ligated on the left spinal cord transected at level of L5 Result Immediate abrupt sustained rise of 18° F in right foot, and immediate gradual sustained rise of 12° F in the left foot. The ligation of the profunda artery occasioned a slower rise to a lower level on the left.

*Experiment 139* Room temperature 70° F femoral artery on the right ligated at the bifurcation of the aorta, and on the left below Poupart's ligament spinal cord transected at the level of L5, with no response on either side, spinal cord transected at the level of L2 with an immediate gradual not sustained rise of 10° F in the left foot and 3° F in the right foot

*Experiment 140* Room temperature 67° F, femoral arteries ligated below Poupart's ligament, spinal cord transected at the level of L6 with a resultant immediate abrupt unsustained (1½ hours) rise of 12° F in both feet Later transection of the cord at the level of L3 brought no response in either foot. There was a rise of temperature after section of the sciatic nerve of 16° F on the right and 6° F on the left Thus dog required a larger dose of amytal than usual

*Experiment 146* Room temperature 68° F femoral arteries ligated below Poupart's ligament. There was no response to transection of the spinal cord at the level of L6, nor L3 Neither was there any response in the sciatic section (bilaterally) This was an old dog with much bronchorrhea, and a deep anesthesia was obtained

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## STUDIES ON THE ACTION OF DIURETICS

### I THE EFFECT OF EUPHYLLIN AND SALYRGAN UPON GLOMERULAR FILTRATION AND TUBULAR REABSORPTION<sup>1</sup>

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Since the appearance in 1887 of von Schroeder's first publication (1) concerning the action of xanthine diuretics there has been much debate regarding the mechanism by which these substances produce diuresis. Similarly there has been considerable controversy as to the manner in which salyrgan and other organic mercury compounds increase the output of urine.

#### REVIEW OF LITERATURE

In the earlier work on xanthine diuresis investigators were chiefly interested in distinguishing between direct action on the renal cells and effects produced through changes in renal circulation. Von Schroeder (1) (2) concluded from his experiments that the effect of caffeine and related substances upon urine output was independent of circulatory changes. Loewi, Fletcher and Henderson (3) held the opposite point of view. They confirmed the observation, first made by Phillips and Bradford (4), that caffeine usually increases the volume of the kidney and considered this as evidence that diuresis was caused by dilatation of the renal blood vessels. Although this observation concerning kidney volume during caffeine diuresis is generally accepted, its significance has been questioned by Gottlieb and Magnus (5) because diuresis sometimes occurs without increase in kidney volume, by Brings and Molitor (6) who concluded that the increase in volume was of too short duration to be significant, and by Cushny (7) who pointed out that increase in kidney volume does not necessarily indicate vasodilatation.

It has been demonstrated by direct measurement that there is usually an increase in renal blood flow following the administration of the xanthine diuretics. However, the evidence seems to indicate that the diuresis is independent of this. Cushny and Lambie (8), E. Schmidt (9), Ozaki (10), and R. Schmidt (11) have reported that the acceleration in rate of blood flow is of short duration and that diuresis may begin before the increase occurs and persist after the flow has returned to normal. Richards and Plant (12) demonstrated that caffeine increased the output of the perfused rabbits' kidney when the rate of blood flow was kept constant, and Gremels (13) (14), working with a Starling heart lung kidney preparation, obtained diuresis with caffeine, theobromine and theocin when increase in rate of flow was prevented by previously dilating the renal vessels to a maximum. Miwa and Tamura (15) found no increase

<sup>1</sup> The major part of this work was reported at the 1931 meeting of The American Society for Clinical Investigation.

in renal blood flow during caffeine diuresis, and Hartwich (16) observed diuresis in the isolated kidney of the frog perfused with Ringer's solution, without increase in rate of perfusion flow Dreyer and Verney (17) have presented evidence that the rate of urine flow is independent of the velocity of blood flow

These observations do not, however, dispose of the possibility that xanthine diuresis is related to changes in the renal vessels Richards and Schmidt (18) observed, during direct microscopic study of the frog's kidney, that caffeine considerably increased the number of functioning glomeruli and the number of patent capillary loops in individual glomeruli Richards and his co-workers (19) (20) (21) have also presented evidence that chemical substances may constrict the vas efferens of the frog's glomerulus to a greater degree than the vas afferens It is conceivable, as Verney and Winton (22) have pointed out, that caffeine increases intraglomerular pressure by dilating the afferent vessels to a greater extent than the efferent vessels, this increase in glomerular capillary pressure not necessarily being accompanied by an increase in rate of blood flow during the whole of diuresis In support of this hypothesis Verney and Winton have shown, in experiments with the heart-lung-kidney preparation, that increase in perfusion pressure and caffeine produces the same changes in the composition of the urine and that caffeine in large doses regularly produces an increase in rate of blood flow through the kidney

Von Schroeder, convinced that the increase in urine flow caused by the purine derivatives was independent of circulatory changes, attributed the diuresis to stimulation of the renal cells to greater secretory activity Gottlieb and Magnus (5), Schmidt (11), Barcroft and Straub (23), Gremels (24), Wohlenberg (25) and others expressed similar views In support of this theory it has been maintained by Barcroft (23) (26) and Gremels (24) that an increase in oxygen consumption occurs However, Miwa and Tamura (15) and Hayman and Schmidt (27) found no evidence of increased metabolism and Masuda (28) observed that caffeine action persisted in the presence of potassium cyanide Wohlenberg (25) stated that the action of weak caffeine and theophyllin solutions on the perfused frog's kidney could be prevented by asphyxiation or narcotization of the metabolic processes occurring in the glomeruli Since larger doses of the diuretics were effective in spite of such asphyxiation and narcotization he was forced to admit the possibility of other factors such as increased permeability of the glomerular membrane

Sobierański (29) reported that indigotate stained the tubular epithelium less than normally during caffeine diuresis, and maintained that the purine derivatives acted by inhibiting reabsorption in the tubules This observation can just as readily be explained on the basis that a more rapid flow of fluid down the tubules afforded less opportunity for diffusion of the dye into the tubule cells Tashiro and Abe (30) repeated Sobierański's experiments and obtained similar results, but Teploff (31) found no evidence that theocin had any influence upon the extent to which the cells of the renal tubules were stained by carmine

Cushny (7) agreed that the purine derivatives had a direct action on the renal cells but suggested on the basis of his "Modern Theory" of urine secretion that the permeability of the capsule was increased and the resistance to filtration thus reduced Brühl (32) conducted experiments with collodion membranes, the pores of which were filled with albumin to simulate the capsule He reported a reversible increase in permeability in the presence of caffeine and considered this due to a change in the dispersion of the protein molecules in the pores

In recent years many investigators have attributed the action of the

xanthine diuretics to extra renal factors Veil and Spiro (33) and Ellinger, Heymann and Klein (34) (35) reported that caffeine increased the capacity of blood serum for ultrafiltration, and proposed that the purines reduced the affinity of the proteins for water Faludi (36) was unable to confirm this observation and Meyer (37) recently reported that euphyllin increased the water binding power of the blood

Asher and his pupils maintain that the xanthine diuretics act by increasing the permeability of the tissues the response on the part of the kidneys being secondary to an increase of chloride and other electrolytes in the blood stream This theory receives its principal support from the observation made by Curtis (38) (39) (40) (41) that the diuretic action of euphyllin is decreased by the simultaneous intraperitoneal injection of isotonic or hypotonic sucrose solution, and is blocked almost completely by a similar injection of distilled water, these injections preventing the slight increase in plasma sodium chloride concentration which was observed in control experiments and which was considered the prime factor in stimulating the kidney to greater activity Hartwich (42) found, on the other hand that the intraperitoneal injection of hypertonic salt solution which presumably withdrew water from the blood, did not prevent euphyllin from producing diuresis Riesen (43) reported that euphyllin increased the electrolyte concentration of an artificial fluid perfused through the intestines of the frog The observations made by Nakao (44) (45) and Raulston (46) are of interest but do not lend much support to the Asher theory

It is difficult to understand how a change in tissue permeability can increase the electrolyte concentration of the plasma It would be more logical to assume that a shift of water and salt from the tissues to the plasma occurred as the result of an increase in hydrogen ion concentration The fact that the xanthine diuretics produce diuresis when the kidneys are isolated from the other tissues (11) (13) (16) (22) makes such an assumption unnecessary Büchler (47) conceded that the "specific diuretics" had a direct renal action since diuresis occurred in the isolated kidney of the frog, but concluded that this renal action played an insignificant rôle because the amount of diuretic necessary to produce the effect was large and the resulting increase in urine output was small

Meyer's report (37) that the colloid osmotic pressure of the blood is increased after the intravenous injection of euphyllin has been referred to above He also expressed the opinion that euphyllin had both a renal and an extra-renal action Molitor and Pick (48) concluded from their experiments that the purines acted through the medium of a center in the hypothalamus which regulated the water exchange.

In the case of the organic mercury compounds most investigators have directed their attention to changes in the chemistry of the blood and urine during diuresis in an effort to prove or disprove the extra renal action of these substances Saxl and Heilig (49) were the first to call attention to the fact that novasurol causes a relative as well as an absolute increase of sodium chloride in the urine This observation has been generally confirmed The concentration of urea, on the other hand, decreases and the absolute amount shows no significant change (Saxl and Heilig (49), Crawford and McIntosh (50), Keith and Whelan (51), Melville and Stehle (52)) Keith and Whelan (51) also reported that there was no appreciable change in the amount of calcium phosphorus or sulphate excreted in the urine while potassium and magnesium showed a slight increase This marked increase in excretion of sodium chloride without significant change in the other constituents of the urine has led many investigators to believe that the primary action of novasurol is an extra renal mobilization of salt and water Sometimes the concentration of chloride in

the urine rises to a value above that in the plasma Möller (53) concluded that this could only be explained by a direct action on the kidneys although he agreed, on the basis of other findings, that mercurial diuretics also had an extra-renal effect

Studies of the blood electrolytes indicate that no significant changes occur following the administration of the mercurial diuretics Nonnenbruch (54) and Möller (53) found no change in the concentration of sodium chloride while Saxl and Heilig (55) reported an initial increase followed quickly by a fall Crawford and McIntosh (50) observed a slight decrease in the concentration of sodium chloride but found no change in the urea Keith and Whelan (51) stated that changes in the blood were inconstant and never marked Noguchi (56) reported that the concentration of potassium in the serum was considerably increased He found a similar increase in potassium during the ingestion of large amounts of water and considered this significant

Many investigators have studied the concentration of protein in the plasma as a measure of the presence or absence of hydremia The reports are conflicting and one gets the impression on reviewing the work that too much significance has often been attached to slight changes Saxl and Heilig (49) in their original publication stated that they often found a decrease in the concentration of protein in the plasma subsequent to the injection of novasurol but that this did not occur regularly Later they reported experiments on normal dogs in which they found an early decrease in concentration with a subsequent rise (55) Bohn (57) (58) also reported a prompt fall in protein concentration after the administration of novasurol This fall lasted only a short time and was followed by an increase in concentration which he considered due to secondary factors Bohn and Saxl and Heilig concluded that the action of novasurol was of an extra-renal nature The findings of Crawford and McIntosh (50) were similar to those quoted above but were interpreted somewhat differently These authors felt that the drug acted not only on the extra-renal tissues but also on the kidneys, the action on the kidneys being the important one during the greater part of the diuresis Serby (59) came to the same conclusion largely on the basis of plasma protein determinations in one case Mühling (60), on the other hand, reported a primary increase and a later fall in concentration and concluded that the primary action was on the kidneys, which responded with an increase in excretion of water and sodium chloride with the result that salt and water were secondarily drawn from the tissues into the blood Nonnenbruch (54) observed an increase in plasma protein concentration Since the erythrocyte count was unchanged he considered this increase absolute and not due to loss of water from the blood Bleyer (61) could demonstrate no constant change A definite fall in concentration occurred in only one of his nine experiments In six of the remaining there were slight increases or decreases not exceeding the limits of error of the method employed

During a certain stage of uranium nephritis Saxl and Heilig (55) observed a marked fall in the plasma proteins, following the injection of novasurol, which persisted for hours This they considered evidence of extra-renal action, the mobilized salt and water remaining in the blood because the damaged kidneys were unable to excrete them Bohn (57) obtained similar results in experiments on nephrectomized rabbits and Möller (53) reported that salyrgan caused a fall in hemoglobin and an increase in serum chloride when administered to animals after removal of the kidneys The normal water metabolism is so disturbed in experiments of this nature that one wonders whether it is justifiable to draw any conclusions

Fehér (62) determined the blood volume by means of the trypan red method and reported a marked increase three hours after the administration of salyrgan. Since a considerable interval necessarily intervened in each instance between the control determination and the determination after salyrgan other factors may have contributed to the changes observed.

Tezner (63) studied the absorption time of subcutaneously injected normal saline solution. He found that the solution was absorbed with somewhat greater rapidity after the administration of novasurol, and interpreted this as evidence that salt and water were being mobilized for excretion. This phenomenon can also be accounted for by a primary renal action.

Recht (64) and Brunn (65) also supported the theory that the mercurial diuretics act primarily on the extra renal tissues. Meyer (66) reported that the colloid osmotic pressure of the blood usually decreased after the injection of salyrgan and then with the onset of diuresis rose again above the control value. In some instances, an immediate increase was observed. He was of the opinion that salyrgan decreased the water binding power of the extra renal tissues but believed that it also had a direct, but less significant, renal action. Fodor (67) concluded that novasurol acted neither directly on the kidneys nor on the tissues, but on a hypothetical center in the medulla which regulated the water and chloride metabolism.

The evidence presented by Gremels (14), Schmidt (11) and Govaerts (68) indicates that the mercurial diuretics act directly on the kidneys. Gremels demonstrated that good diuresis could be obtained from salyrgan and novasurol in the Starling heart lung kidney preparation, and Schmidt observed an increase in urine flow from the kidneys of frogs perfused with colloid free solutions. Govaerts attacked the problem in a very ingenious manner by transplanting kidneys from one animal into the neck of another. When the kidneys from an animal previously given an intravenous dose of novasurol were connected with the circulation of an animal which had received no diuretic, the transplanted kidneys produced an increased amount of urine while the animal's own kidneys continued to excrete urine at a normal rate. When the converse experiment was tried, the transplanted kidneys in the neck put out a normal amount of urine while the animal's own kidneys showed a diuresis.

No correlation between novasurol or salyrgan diuresis and blood flow through the kidneys was found by Gremels, Schloss (69) or Schmidt. An increase in oxygen consumption was reported by Gremels, who concluded that these substances acted directly on the kidneys by stimulating secretion. Kulcke (70) expressed the view that novasurol acted on the tubule cells, small amounts stimulating the secretory function, larger amounts injuring and destroying the cells, as in bichloride poisoning.

From this review of the literature it is obvious that further evidence regarding the mechanisms by which these diuretics increase the output of urine is desirable.

In 1926 Rehberg (71) proposed a method for measuring glomerular filtration and tubular reabsorption. This method is based upon the premises that urine is formed in mammals by a process of filtration and reabsorption and that creatinine is a non threshold substance and hence is not reabsorbed in the tubules. These premises are well substantiated by the work of Richards, Rehberg, Ekehorn (76) and others to whose publications the reader is referred. From this starting point Rehberg



reasoned that the concentration of creatinine in the urine times the urine volume must equal the concentration of creatinine in the glomerular filtrate times the volume of the glomerular filtrate. Since glomerular fluid is a protein-free filtrate of the plasma, the concentration of creatinine in the plasma may be substituted for the concentration of creatinine in the glomerular filtrate. By parallel determinations of creatinine in the plasma and in the urine it is thus possible to calculate the amount of glomerular fluid formed per unit of time. The amount of reabsorption occurring in the tubules is then determined by subtracting the urine volume from the amount of glomerular filtrate.

In the experiments reported below, this method of Rehberg has been employed to investigate the influence of euphyllin and salyrgan upon glomerular filtration and tubular reabsorption. Chrometzka and Unger (72) have recently published the results of similar experiments.

#### PROCEDURE

The experiments were performed upon eight female dogs weighing between ten and thirteen kilos. Food was withheld from the animals for fifteen hours preceding the experiment. Three hours before the beginning of the experiment proper 3 grams of creatinine in 50 cc of water were given by stomach tube. This sufficed to raise the initial concentration of creatinine in the plasma to values ranging from 5 to 10 or more mgm per 100 cc and thus made the determinations more accurate. During the course of the experiment the amount of creatinine in the plasma steadily decreased. Hence a fairly accurate average concentration for each period of observation could be obtained. The animals were allowed no water for at least one hour preceding the experiment. At the end of this period the absorption of any water previously present in the alimentary tract had, as a rule, produced its maximum effect upon the urine output. The fluid intake preceding this period of abstinence was varied considerably in order to vary the initial urine volume. In some instances water was withheld entirely except for the small amount given along with the creatinine. In other experiments water was permitted *ad libitum*. When large initial urine volumes were desired, water was given by stomach tube in amounts as high as 200 cc per hour.

During the experiment proper urine collections were made for successive periods before and after the administration of the diuretic to be studied. These collections were made directly into accurately graduated cylinders by means of a stiff rubber catheter inserted into the bladder through the urethra. The catheter was left in place throughout the experiment. Toward the end of each period it was moved about to assure as complete emptying of the bladder as possible.

In some experiments blood samples were obtained at the middle of each period of urine collection. Usually, however, fewer blood specimens

were taken and the concentration of creatinine corresponding to the middle of each period was then obtained by graphing the known values. The creatinine determinations were made by the method of Folin (73). Both euphyllin and salyrgan were given intravenously. The saline injections were made by the gravity method.

As a rule, two or more control periods of ten minutes each were obtained before the diuretic was administered. When the initial urine volume was quite small a single long control period was used. When the urine volume showed a tendency to increase during the control periods the administration of the diuretic was postponed until the urine output began to decrease. In addition to these control periods preceding the administration of diuretics several control experiments were performed.

The results of the experiments are presented in the accompanying tables and graphs. For purposes of comparison the urine volumes and rates of glomerular filtration are expressed in terms of cubic centimeters per minute. The amount of fluid reabsorbed in the tubules per minute is expressed as the per cent of the corresponding volume of filtrate.

### Controls

The variations in glomerular filtration and in tubular reabsorption which occurred during successive periods of observation under the conditions of the experiment are shown in Table 1. The changes in filtration

TABLE 1  
*Spontaneous variations in glomerular filtration and tubular reabsorption*

Dog number	Time	Urine volume	Glomerular filtration	Reabsorption of water in tubules	Change in glomerular filtration		Change in reabsorption	
					Increase	Decrease	Increase	Decrease
		cc. per minute	cc. per minute	per cent	cc. per minute	cc. per minute	per cent	per cent
100	2.27-2.37	2.2	76.0	97.1				
	2.37-2.47	1.3	62.5	97.9		13.5	0.8	
	2.47-2.57	0.9	63.0	98.5	0.5		0.6	
	2.57-3.07	0.95	72.0	98.6	9.0		0.1	
	3.07-3.17	0.98	66.5	98.5		5.5		0.1
	3.17-3.27	1.02	58.5	98.3		8.0		0.2
101	1.56-2.06	3.4	66.5	94.9				
	2.06-2.16	2.35	54.0	95.6		12.5	0.7	
	2.16-2.26	1.55	56.0	97.2	2.0		1.6	
	2.26-2.36	1.45	56.5	97.4	0.5		0.2	
	2.36-2.46	1.25	50.5	97.5		6.0	0.1	
102	2.12-2.22	3.6	68.5	94.7				
	2.22-2.32	4.1	71.5	94.2	3.0			0.5
	2.32-2.42	3.6	62.0	94.2		9.5		
	2.42-2.52	3.1	55.0	94.3		7.0	0.1	
	2.52-3.02	2.9	51.5	94.4		3.5	0.1	

TABLE 1 (continued)

Dog number	Time	Urine volume	Glomerular filtration	Reabsorption of water in tubules	Change in glomerular filtration		Change in reabsorption	
					Increase	Decrease	Increase	Decrease
		cc. per minute	cc. per minute	per cent	cc. per minute	cc. per minute	per cent	per cent
102	2 08-2 18	1 8	59 0	96 9				
	2 18-2 28	1 35	53 0	97 4		6 0	0 5	
	2 28-2 38	1 2	50 5	97 6		2 5	0 2	
103	2 06-2 16	4 7	142 0	96 7				
	2 16-2 26	5 1	137 0	96 3		5 0		0 4
	2 26-2 36	4 1	129 5	96 8		7 5	0 5	
	2 36-2 46	2 45	121 5	97 9		8 0	1 1	
103	2 14-2 25	1 36	101 0	98 7				
	2 25-2 35	0 9	75 0	98 8		26 0	0 1	
	2 35-2 45	0 35	56 0	99 4		19 0	0 6	
104	1 06-1 16	1 55	81 5	98 1				
	1 17-1 27	1 42	64 5	97 8		17 0		0 3
	1 27-1 37	1 35	64 0	97 9		0 5	0 1	
	1 37-1 47	1 0	62 5	98 4		1 5	0 5	
104	2 05-2 25	0 18	91 0	99 8				
	2 25-2 45	0 17	81 0	99 8		10 0		
	2 45-3 05	0 16	85 0	99 8	4 0			
105	2 15-2 25	2 1	56 5	96 3				
	2 25-2 35	2 25	58 5	96 1	2 0			0 2
	2 35-2 45	1 8	57 0	96 8		1 5	0 7	
	2 45-2 55	1 64	58 0	97 2	1 0		0 4	
105	2 25-2 35	3 3	96 0	96 5				
	2 35-2 45	3 45	89 5	96 1		6 5		0 4
	2 45-2 55	3 55	96 5	96 3	7 0		0 2	
106	1 47-2 02	0 5	35 0	98 6				
	2 02-2 17	0 6	22 0	97 3		13 0		1 3
	2 17-2 37	0 25	20 0	98 7		2 0	1 4	
	2 37-2 58	0 12	34 0	99 6	14 0		0 9	
106	3 32-3 42	3 35	80 0	95 8				
	3 44-3 55	3 1	75 0	95 8		5 0		
	3 55-4 05	2 75	71 0	96 1		4 0	0 3	
	4 05-4 15	2 4	69 5	96 5		1 5	0 4	
106	2 03-2 13	2 3	69 5	96 7				
	2 13-2 23	2 0	66 0	96 9		3 5	0 2	
	2 23-2 33	1 85	55 0	96 6		11 0		0 3
	2 33-2 43	1 07	54 0	98 0		1 0	1 4	
107	2 00-2 15	1 73	44 0	96 1				
	2 15-2 30	1 93	41 0	95 3		3 0		0 8
	2 30-2 45	1 33	46 5	97 1	5 5		1 8	
	2 45-3 00	1 33	43 0	96 9		3 5		0 2

represent not only actual increases or decreases in rate of formation but also the variations resulting from experimental errors such as those occurring in the creatinine determinations. Of primary interest are increases in filtration and decreases in reabsorption, the two factors which tend to augment urine output. Increases in filtration occurred infrequently and seldom were significant. The maximum observed was 14 cc. per minute. Decreases in the percentage of reabsorption in the tubules were also uncommon. The largest decrease was 13. In the majority of instances, decreases in filtration and increases in percentage reabsorption occurred, both of these factors tending to diminish the urine volume.

### *Euphyllin experiments*

The experiments with euphyllin may be divided into two groups, those in which diuresis was obtained, and those in which no increase in urine output occurred. The results of the former group are presented in Table 2 and Figure 1.

Euphyllin diuresis was characteristically associated with an increase in the calculated rate of glomerular filtration. In a few experiments the maximum rate of filtration immediately preceded the maximum urine volume. In experiments 57 and 58 diuresis persisted after the rate of filtration had returned to normal. Changes in reabsorption were variable. In experiments 1, 4 and 14 the percentage reabsorption was definitely increased. In four other experiments (34, 50, 63 and 64) there was no significant change during the course of the diuresis. In only three instances were there decreases in reabsorption which definitely exceeded the maximum observed in the controls. However, the occurrence of decreases was more frequent than in the control experiments and in a number of instances decreased reabsorption contributed considerably to the increase in urine output.

The urine output after the intravenous injection of 2 cc. of euphyllin only twice exceeded 2 cc. per minute. As a rule diuresis began within five minutes after the administration of the drug.

Practically all of the failures to obtain diuresis with euphyllin occurred in experiments in which the urine volume to begin with was 1 cc. per minute or greater. In fifteen of seventeen experiments in which the control urine volumes were below 1 cc. per minute the injection of euphyllin was followed by diuresis. In four experiments with initial urine volumes ranging between 1 cc. and 1.4 cc. per minute, the response to euphyllin was variable. In six experiments with control urine volumes greater than 1.5 cc. per minute, there was a decrease in urine volume following the injection of euphyllin. In the latter experiments the rate of filtration increased within ten to twenty minutes after the administration of the drug, but this rise in filtration was more than counteracted by a considerable increase in the percentage of reabsorption in the tubules.

TABLE 2  
*Effect of euphyllin upon glomerular filtration and tubular reabsorption*

Experiment number	Dog number	Urine volume			Glomerular filtration			Reabsorption of water in tubules		
		Control before diuretic	Maximum after diuretic	Increase	During control period	During maximum diuretics	Change	During control period	During maximum diuretics	Change
		cc. per minute	cc. per minute	cc. per minute	cc. per minute	cc. per minute	cc. per minute	per cent	per cent	per cent
34	104	0.1	0.6	0.5	36	169	+133	99.7	99.6	-0.1
47	105	0.19	2.0	1.81	15	76	+61	98.8	97.4	-1.4
57	106	0.2	1.4	1.2	85	145	+60	99.8	99.0	-0.8
64	107	0.2	0.95	0.75	25	144	+119	99.3	99.3	0
61	107	0.2	4.5	4.3	56	200	+144	99.6	97.7	-1.9
12	105	0.22	1.3	1.08	36	75	+39	99.3	98.2	-1.1
58	106	0.24	0.86	0.62	105	172	+67	99.8	99.5	-0.3
2	100	0.27	2.1	1.83	58	80	+22	99.5	97.4	-2.1
50	106	0.36	0.74	0.38	160	211	+51	99.8	99.6	-0.2
3	101	0.4	1.2	0.8	41	70	+29	99.0	98.3	-0.7
38	105	0.5	1.3	0.8	51	71	+20	99.0	98.2	-0.8
63	107	0.65	1.75	1.1	39	119	+80	98.3	98.5	+0.2
16	105	0.67	1.85	1.18	40	60	+20	98.3	97.0	-1.3
14	105	0.75	1.5	0.75	32	101	+69	97.6	98.5	+0.9
4	100	0.8	1.45	0.65	39	109	+70	97.9	98.7	+0.8
1	101	1.05	1.45	0.4	38	82	+44	97.3	98.2	+0.9
20	104	1.4	2.45	1.05	45	65	+20	96.9	96.2	-0.7

0.48 gram of euphyllin was administered in each of the experiments

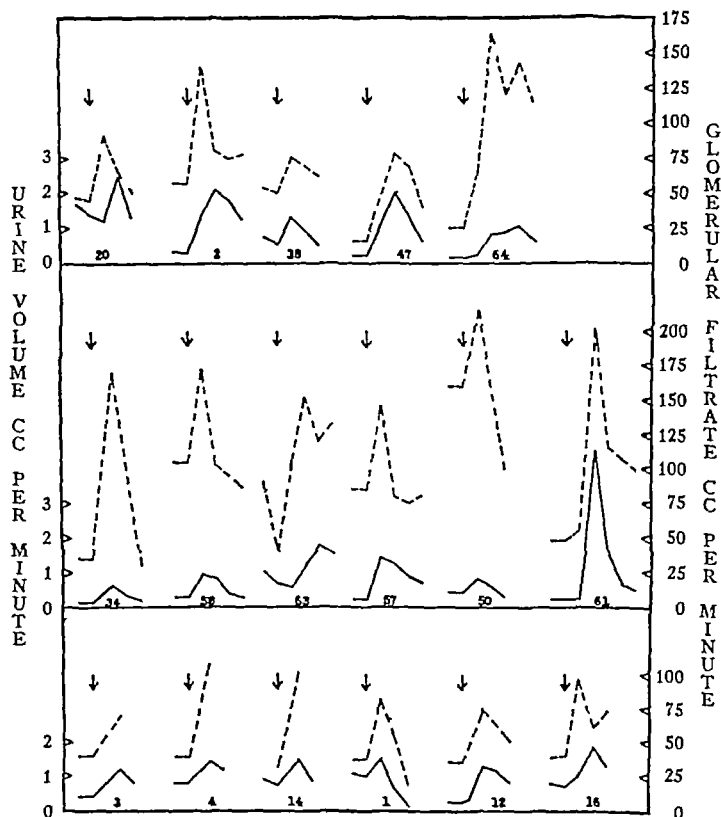


FIG 1 RATE OF GLOMERULAR FILTRATION DURING EUPHYLLIN DIURESIS

The arrows indicate when euphyllin was injected. Solid line = urine volume. Broken line = glomerular filtrate.

This is illustrated by Figure 3 in which one of the experiments is presented graphically.

#### *Salyrgan experiments*

The results obtained with salyrgan (see Table 3 and Figure 2) are in sharp contrast to those given by euphyllin. The percentage reabsorption in the tubules was significantly decreased in all but one instance, the decreases ranging from 1.6 to 4.6. A definite increase in filtration, on the other hand, occurred in only four of the fourteen experiments and in only one instance (experiment 6) played any significant rôle in the production of diuresis.

Salyrgan was effective even when the control urine volume was as high as 3.5 cc per minute. Rates of urine excretion as high as 5, 6.8 and 9.4 cc per minute were obtained. The response to salyrgan is less rapid than that to ephyllin. In the majority of experiments diuresis began 15 to 20 minutes after the intravenous administration of the drug. There were only two instances in which salyrgan failed to cause diuresis.

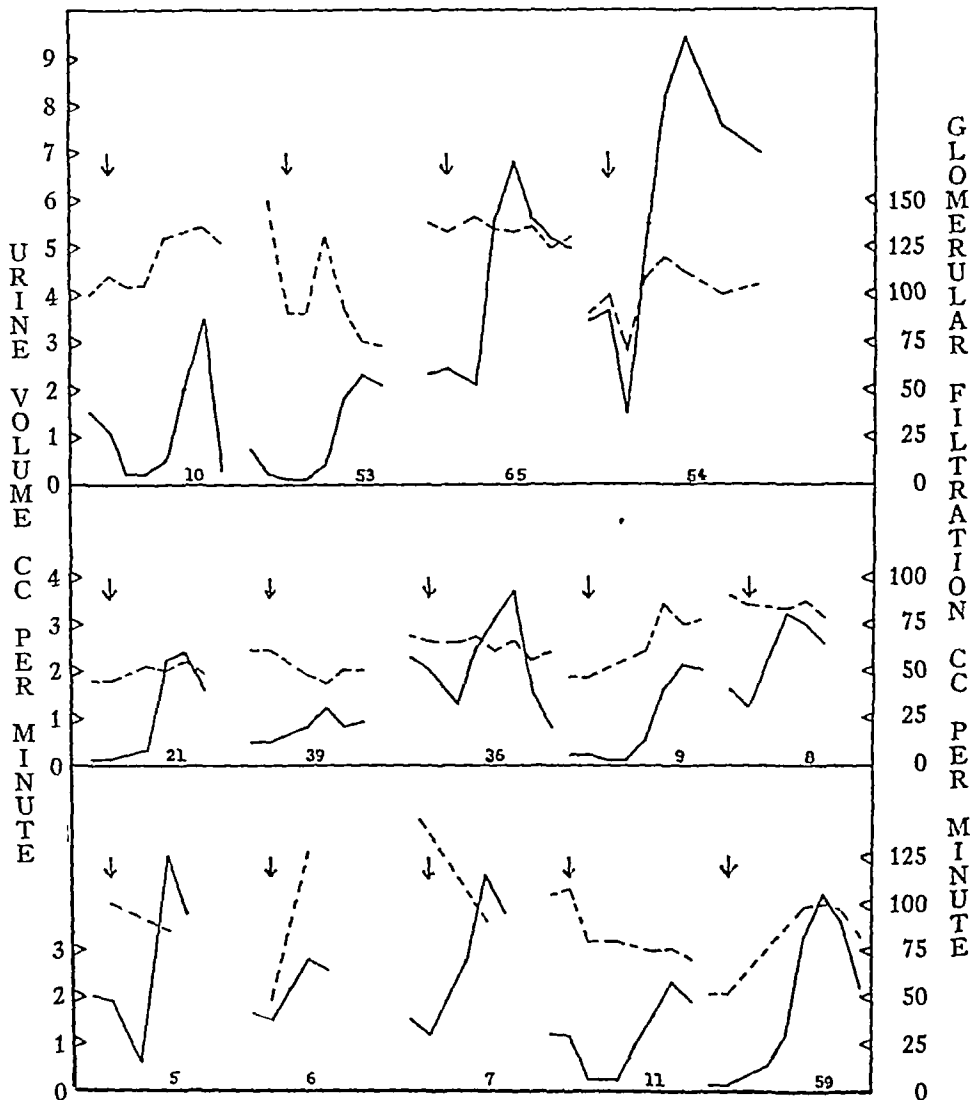


FIG. 2 RATE OF GLOMERULAR FILTRATION DURING SALYRGAN DIURESIS

The arrows indicate when salyrgan was injected. Solid line = urine volume. Broken line = glomerular filtrate.

#### *Intravenous normal saline solution*

Because many investigators have maintained both in the case of ephyllin and in the case of salyrgan that diuresis is the result of a primary

extra-renal mobilization of salt and water the effect of normal saline solution given intravenously was studied for purposes of comparison. The amount of saline and the rate of injection were varied considerably. See Table 4.

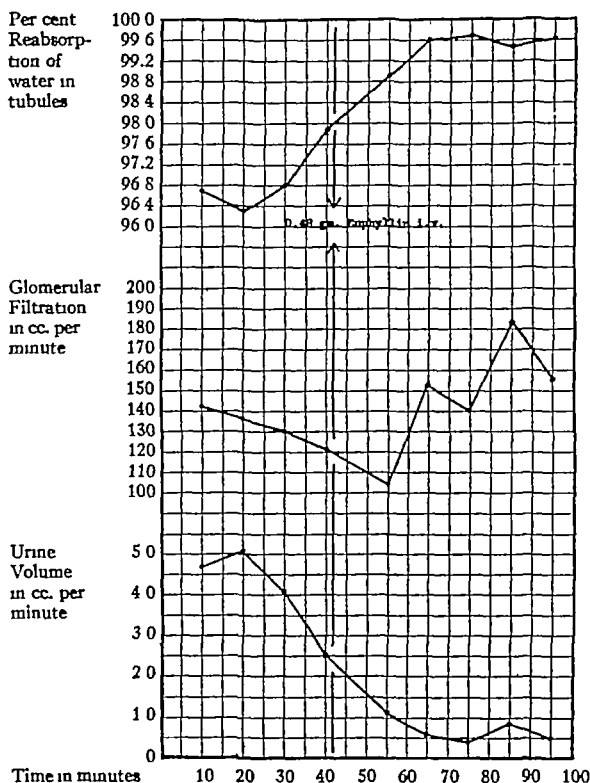


FIG 3 EFFECT OF EUPHYLLIN DURING WATER DIURESIS

The almost constant increase in rate of glomerular filtration following the injection of saline was outstanding in these experiments. The effect upon reabsorption varied with the amount and the rate of injection of saline. With the smaller amounts decreases in reabsorption were infrequent and seldom significant. When saline was injected continuously at fairly rapid rates, larger decreases in percentage reabsorption occurred. In the latter experiments the increases in filtration always appeared first. As the injection was continued the rate of filtration



TABLE 3  
*Effect of salyrgan upon glomerular filtration and tubular reabsorption*

Experi- ment number	Dog number	Urine volume			Glomerular filtration			Reabsorption of water in tubules		
		Control before diuretic	Maximum after diuretic	Increase	During control period	During maximum diuresis	Change	During control period	During maximum diuresis	Change
		cc per minute	cc per minute	cc per minute	cc per minute	cc per minute	cc per minute	per cent	per cent	per cent
39	105	0.5	1.2	0.7	62	44	-18	99.2	97.3	-1.9
11	105	1.15	2.3	1.15	108	76	-32	98.9	96.9	-2.0
36	105	2.0	3.7	1.7	66	66	0	97.0	94.4	-2.6
59	106	0.1	4.2	4.1	52	99	+47	99.8	95.8	-4.0
21	104	0.11	2.4	2.29	44	55	+11	99.7	95.6	-4.1
9	104	0.125	2.1	1.97	46	76	+30	99.7	97.2	-2.5
53	106	0.23	2.3	2.07	151	75	-76	99.8	96.9	-2.9
10	104	1.1	3.5	2.4	111	136	+25	99.0	97.4	-1.6
7	103	1.2	4.6	3.4	146	91	-55	99.1	95.0	-4.1
8	103	1.25	3.2	1.95	85	83	-2	98.5	96.1	-2.4
6	103	1.53	2.8	1.27	48	129	+81	96.8	97.9	+1.1
5	102	1.9	5.0	3.1	99	86	-13	98.0	94.2	-3.8
65	107	2.4	6.8	4.4	134	133	-1	98.2	94.9	-3.3
54	106	3.55	9.4	5.85	97	114	+17	96.3	91.7	-4.6

0.5 cc of salyrgan was used in experiments 11, 36 and 39. In all other experiments 1.0 cc of the drug was administered.

TABLE 4  
*Effect of intravenous saline upon glomerular filtration and tubular reabsorption*

Experiment number	Dog number	Dose	Urine volume			Glomerular filtration			Reabsorption of water in tubules		
			During control period	During maximum diuretics	Increase	During control period	During maximum diuretics	Change	During control period	During maximum diuretics	Change
			cc. per minute	cc. per minute	cc. per minute	cc. per minute	cc. per minute	cc. per minute	per cent	per cent	per cent
43	105	100 cc. in 5 minutes	0.42	0.85	0.43	64	97	+33	99.3	99.1	-0.2
46	105	100 cc. in 8 minutes	0.15	0.15	0.00	10	66	+56	98.5	99.8	+1.3
49	105	150 cc. in 5 minutes	0.28	0.6	0.32	34	69	+35	99.2	99.1	-0.1
44	105	150 cc. in 10 minutes	0.3	1.05	0.75	18	100	+82	98.3	98.9	+0.6
51	106	150 cc. in 15 minutes	0.28	2.1	1.82	100	119	+19	99.7	98.2	-1.5
52	106	150 cc. in 50 minutes	0.14	2.3	2.16	67	103	+36	99.8	97.7	-2.1
56	106	Constant 5 cc. per minute	0.35	2.0	1.65	56	103	+47	99.4	98.0	-1.4
29	105	Constant 5 cc. per minute	0.13	2.25	2.12	22	72	+50	99.4	96.9	-2.5
28	104	Constant 10 cc. per minute	0.1	5.4	5.3	67	119	+52	99.8	95.4	-4.4
23	105	Constant 10 cc. per minute	0.11	2.2	2.09	30	57	+27	99.6	96.2	-3.4
26	104	Constant 10 cc. per minute	2.9	4.0	1.1	51	47	-4	94.4	91.5	-2.9

tended to subside while reabsorption decreased. In experiment 26 the rate of filtration, after an initial rise, dropped below the control period level.

#### COMMENT

In the experiments with salyrgan, diuresis was characteristically associated with a decrease in tubular reabsorption. Chrometzka and Unger (72) made the same observation. The fact that there is no resemblance between salyrgan and intravenous normal saline solution, so far as effect upon filtration is concerned, is opposed to the view that the response of the kidneys to this mercurial diuretic is secondary to an extra-renal mobilization of salt and water. The observations of Gremels and Govaerts, referred to above, also indicate a direct action on the kidneys. Since larger doses of mercury injure and destroy the epithelium of the renal tubules, it seems logical to assume that this decrease in tubular reabsorption is due to a depressant action upon the tubule cells.

Chrometzka and Unger performed three experiments with diuretin and found no change in the rate of glomerular filtration. In the experiments reported in this paper, however, an increase in rate of glomerular filtration was characteristic of euphyllin diuresis. This finding is consistent with the direct observation of Richards and Schmidt that caffeine increases the number of functioning glomeruli and the number of functioning capillary loops in individual glomeruli. It is also in harmony with Bieter's observation (74) that caffeine and theophyllin do not produce diuresis in fish with aglomerular kidneys.

Since the intravenous injection of normal saline solution also increases the rate of glomerular filtration it is impossible to conclude from these experiments alone whether xanthine diuretics act primarily upon the tissues or upon the renal vessels. The fact that these diuretics are effective in increasing the urine output of the isolated kidney is definitely opposed to the former view. The principal objection to the latter theory has been the lack of correlation between xanthine diuresis and rate of blood flow through the kidney. This objection loses its significance if we assume, as Verney and Winton (22) have proposed in the case of caffeine, that the xanthine diuretics may, under suitable circumstances, dilate the vas afferens to a greater extent than the vas efferens and thus increase the glomerular capillary pressure, the glomerular capillary surface and the rate of glomerular filtration without necessarily increasing the rate of blood flow through the kidney.

Changes in reabsorption following the injection of euphyllin are inconstant. The moderate decreases occurring in many experiments may well be related to increases in the number of patent capillaries in individual glomeruli, larger amounts of fluid flowing down the corresponding tubules more rapidly and lessening the chances for reabsorption.

The diuretic response to salyrgan is considerably greater than that to

euphyllin This difference is related to the relative effect of increases in filtration and decreases in reabsorption upon urine volume Even large increases in filtration can not per se produce large urine volumes whereas relatively small decreases in reabsorption can cause considerable diuresis

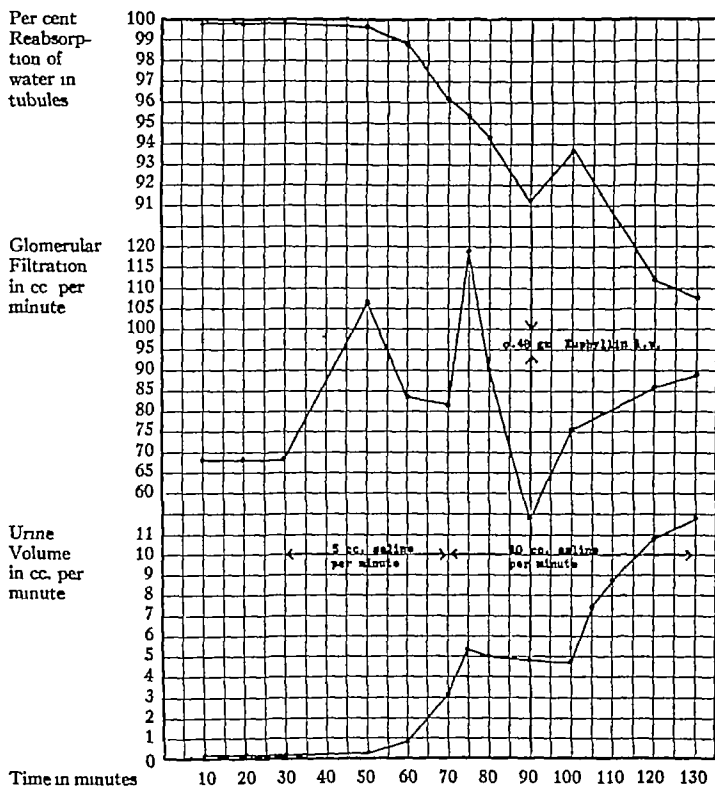


FIG 4 EFFECT OF EUPHYLLIN DURING CONTINUOUS INTRAVENOUS INJECTION OF NORMAL SALINE SOLUTION

Fremont-Smith and his associates (75) found that water diuresis in man was not associated with an appreciable increase in the amount of glomerular filtration Chrometzka and Unger (72) have made the same observation In the experiments herein reported, the rates of filtration associated with high control urine volumes, produced by forcing large amounts of water, were moderately higher than the rates of filtration associated with low control urine volumes The average rate of filtration

during 38 periods with urine volumes below 1 cc per minute was 54 cc per minute, while the average rate for 10 periods with urine volumes between 3 and 4 cc was 78 cc per minute

The amount of reabsorption, on the other hand, is considerably reduced during water diuresis. As the diuresis subsides the percentage reabsorption mounts rapidly. This rise in percentage reabsorption would seem to be the significant factor in the failure of euphyllin to increase urine volume under these conditions (see Figure 2). Filtration is increased, but the rising percentage of reabsorption in the tubules more than counteracts the increase in rate of filtration. Presumably if water were administered at a constant rate so as to maintain the percentage of reabsorption at a level, euphyllin would be capable of increasing

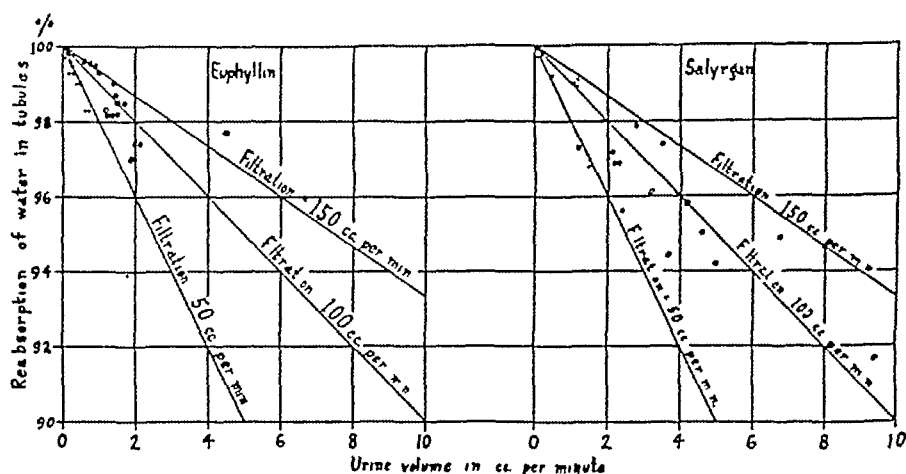


FIG 5 GRAPHIC COMPARISON OF THE EFFECT OF EUPHYLLIN AND SALYRGAN UPON GLOMERULAR FILTRATION AND TUBULAR REABSORPTION

Control readings ●

After diuretic ○

the urine volume still further. Euphyllin is effective when the urine output is high as a result of the continuous injection of normal saline solution (Figure 4). It is in fact more effective than usual under these circumstances, because the low percentage of reabsorption in the tubules makes the resulting increase in filtration more significant. Similar results were obtained in two other experiments of this type.

These experiments are of interest to the clinician because they may lead to a better understanding of the clinical results obtained with these drugs. Clinically it has been found that the xanthine diuretics are chiefly of value in hastening the elimination of cardiac edema after digitalis and bed rest have improved the circulation. With improvement in circulation in cardiac failure salt and water previously stored in the tissue spaces are brought to the kidneys for excretion. Under these conditions, as we have seen experimentally, the increase in filtration produced by the purine

derivatives is more effective than usual because reabsorption is taking place to a lesser degree than normal. In other types of edema (hepatic and renal) the xanthine diuretics are of little help. Increase in filtration, especially when only of a transient nature, is by itself of little value in augmenting the urine output.

The mercurial diuretics, on the other hand, are effective in all three of the above mentioned types of edema. The effectiveness of salyrgan and novasurol in the case of cardiac edema is readily understandable. It is more difficult, however, to account for the removal of accumulations of fluid resulting from portal obstruction and from depletion of the plasma proteins. Presumably the loss of water from the plasma resulting from the administration of these mercurial diuretics is sufficient to secondarily attract fluid from the tissue accumulations into the blood. With our present information, however, one can do little more than speculate in this regard. Further observations, both on the experimental animal and on patients with edema, are necessary before one can draw any definite conclusions.

#### SUMMARY

Rehberg's method of calculating the amount of filtration occurring in the glomeruli and the amount of reabsorption taking place in the tubules was used to compare the diuretic action of euphyllin and salyrgan in the dog.

During euphyllin diuresis the calculated amount of glomerular filtration is consistently increased while tubular reabsorption shows no constant change.

Salyrgan diuresis is characterized by a considerable decrease in the percentage of fluid reabsorbed in the tubules. The amount of filtration is seldom increased significantly.

The intravenous injection of normal saline solution characteristically increases the rate of glomerular filtration. The effect upon reabsorption varies with the amount of saline and the rate of injection.

The mechanisms by which mercurial and xanthine diuretics produce diuresis are discussed in the light of these findings and of the evidence available in the literature.

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# THE LUNG VOLUME AND ITS SUBDIVISIONS

## I METHODS OF MEASUREMENT

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In perhaps no realm of physiology is there a more confusing medley of terms than in that which deals with the lung volume and its various subdivisions. Not only does the meaning of many terms vary with the user but there is an unfortunate superfluity and synonymy of the terms themselves.

*Residual air* as first described by Davy (1800) is the amount of air remaining in the lungs after the fullest possible expiration.

*Vital capacity* as described by Hutchinson (1846) is the amount of air that can be expired from the fullest inspiration to the fullest expiration.

*Total lung volume* or total capacity is the sum of residual air and the vital capacity.

*Mid capacity* as defined by Panum (1868) is the amount of air in the lungs at a point mid way between normal inspiration and expiration, this level being referred to as the mid position or mid point or even vital respiratory level. Siebeck (1910) and others prefer to describe the mid capacity as a quantity synonymous with the functional residual air (vide infra).

*Functional residual air* as described by Lundsgaard and Schierbeck (1923) is that amount of air remaining in the lungs after a normal expiration. This quantity has also been called the mid capacity or normal capacity, and the level has been named the resting respiratory level, the expiratory level, the mid position or mid point.

*Complemental air* has been variously described as the amount of air inspired from the mid position to maximum inflation, or from the height of a normal inspiration to maximum inflation.

*Reserve air* or *supplemental air* has been variously described as the air expired from the mid position to maximum deflation or from the end of a normal expiration to maximum deflation.

*Tidal air* is the quantity of air expired by a breath of average depth.

Were it not for the confusion that already exists we would hesitate to offer any further addition to this classification but simplification necessarily involves some minor changes in definitions. The terms vital capacity and residual air have stood the test of time and we would not modify them. However, they are the product of respiratory gymnastics and surely it is important to establish some simple nomenclature with respect to the level at which we normally breathe. A glance at any

series of respiratory tracings, as taken in the routine estimation of the basal metabolic rate, shows clearly that the constant respiratory level is at the end of expiration. The inspiratory level and mid-capacity vary with the depth of inspiration, a variation which is often considerable both from breath to breath and from minute to minute. From a functional point of view also, it is the amount of air remaining in the lungs after expiration (the functional residual air) that we have to ventilate. Without entering into a discussion of the Hering Breuer reflex it can be safely said that there is much evidence that it is from the expiratory level (resting respiratory level) that the respiratory cycle has its initiation. Also it is at this level that there occurs the maximum pause during the respiratory cycle. To inflate the lungs above it one group of muscles is used, while to deflate the lungs below it an entirely different group is used. It would seem then that, from both practical and theoretical considerations, any measurements involving the amount of air normally present in the lungs should be based on the functional residual air or the resting respiratory level. On this basis we have adopted the nomenclature depicted in Figure 1. The complemental and reserve airs are

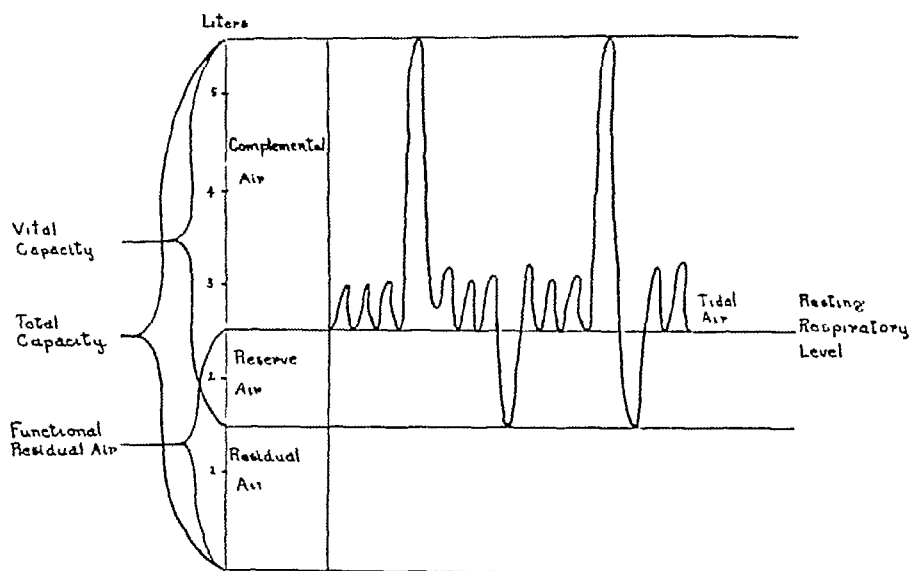


FIG 1 THE LUNG VOLUME AND ITS SUBDIVISIONS

measured from the resting respiratory level and we agree with Anthony (1927 and 1930) that these should be measured separately, but are equally convinced that the vital capacity should also be measured independently, as in its original definition by Hutchinson. The reasons for this are obvious when we consider those cases where there is any impairment of pulmonary elasticity. Two tracings of the vital capacity in such a case are shown in Figure 2 and it is obvious that if we divide the vital capacity into complemental and reserve air we are confronted with

the absurdity of having a negative quantity for the latter. Actually when the reserve air was measured separately in this case it amounted to some 300 cc. The significance of this type of tracing will be discussed in a subsequent communication but we believe that such a discrepancy between the sum of the complemental and reserve airs measured in this way, and the vital capacity, represents the earliest sign of impairment of pulmonary elasticity. This feature can only be brought out if the tracings are taken in the manner described.

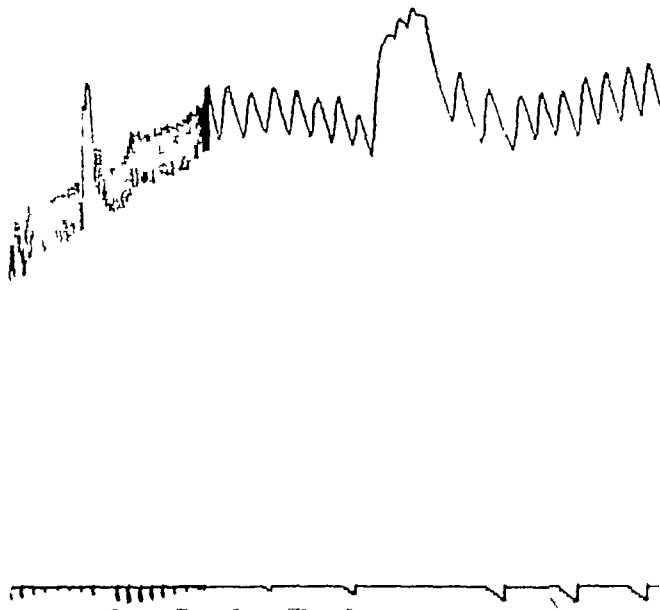


FIG 2 THE VITAL CAPACITY IN A CASE OF ADVANCED EMPHYSEMA  
Time marker 5 seconds

By using this nomenclature a complete analysis of the lung volume and its functional and artificial subdivisions can be made, by stating only 5 quantities. (a) The complemental air, reserve air and vital capacity, (b) the tidal air, (c) the residual or functional residual air. These three groups will be considered separately.

*Measurement of the complemental air, reserve air and vital capacity*

It is obvious that to measure the complemental and reserve air, some method involving the graphic registration of respiration must be used

(Figure 1) By ordinary graphic spirometric methods the volume of air displaced by forced inspiration or expiration can be measured accurately to within 5 or 10 cc, and yet under even carefully controlled conditions we have found these amounts to vary by several hundred cc in duplicate measurements on the same subject

The expulsion of the reserve air is the result of a voluntary unnatural effort and as such can be profoundly influenced by many factors such as posture (Panum (1868), Bohr (1907), Hasselbach (1908), Wilson (1927) and Livingstone (1928)), fatigue (Peabody and Sturgis (1921)), respiratory resistance (Bittorf and Forscbach (1910), Bass (1925) and Thiel (1929)), and various external stimuli (Bittorf and Forscbach (1910)) Even when these factors were carefully excluded<sup>1</sup> we have found variations of over 300 cc in both normal and pathological subjects The same range of variation was found in measurements of the complementary air and the vital capacity (Table I), and as no correlation could be found between

TABLE I  
*Variations in the vital capacity and its subdivisions*

Quantity measured	Group	Number of cases	Number of observations	Average deviation from individual mean	Maximum deviation from individual mean
Vital capacity	Normals	4	28	cc ±110	cc 314
	Emphysema	5	19	± 89	300
	Cardio-respiratory	10	33	±115	403
Reserve air	Normals	4	37	± 78	318
	Emphysema	5	27	± 76	225
	Cardio-respiratory	10	37	± 63	371
Complemental air	Normals	4	32	±109	316
	Emphysema	5	29	± 62	187
	Cardio-respiratory	10	40	±115	350

the variation of these three quantities and they do not permit of statistical analysis, one must conclude that they were due to fortuitous changes in muscular effort rather than changes in the resting respiratory level Indeed this is the only possible explanation since any series of reserve and complementary air estimations made at say  $\frac{1}{2}$  minute intervals show the same variations, although the resting respiratory level may remain a perfectly even base line Such a variation in a voluntary and almost violent respiratory effort as is required for the complete expulsion of the

<sup>1</sup> Subject rested for 15 minutes in recumbent position, measurements then made with arms by side, shoulders flat on bed, and one pillow supporting head

reserve air or indraught of the complemental air is indeed to be expected from a general physiological point of view and necessarily reflects fortuitous variations in the residual air and total capacity

### *Measurement of the tidal air*

The measurement of the tidal air is a very simple physical problem and so many accurate methods have been described that any discussion would be pointless. The same may be said of any discussion of changes in the tidal air due to central and peripheral stimuli. It need only be emphasized that the tidal air is a quantity which varies from breath to breath and only an average over a stated period of time has any real meaning.

### *Measurement of residual or functional residual air*

The number of methods described for the measurement of the residual or functional residual air approaches the number of papers published on this subject, a total of 47 having been reviewed. Although there are obvious fallacies in many of the methods and equally obvious incompatibilities in the results produced, we have been unable to find any critical review of the subject. Before describing the method which we have developed we propose to present some theoretical and experimental criticism of those methods commonly in use. These divide themselves into three main groups

- (1) The so-called pneumatometric method,
- (2) Gas dilution with forced breathing,
- (3) Gas dilution without forced breathing

The principles underlying these methods will be criticised separately without entering into the minor modifications which have been proposed by almost every worker in this field.

### I PNEUMATOMETRIC METHOD

Originally described by Pflüger (1882) this method is based on Boyle's Law, which states that the volume of a gas varies in inverse proportion to the pressure to which it is subjected. The subject is placed in an air-tight cabinet, breathing through a mask or mouthpiece connected with an opening in the cabinet. The air displaced from the cabinet by the respiratory expansion and contraction of the chest is graphically recorded by means of a spirometer. Expiration is suddenly completely obstructed and the subject told to make an expiratory or inspiratory effort against this absolute resistance. The positive or negative pressure so generated in the respiratory tract is recorded by a mercury manometer and the change in the volume of air in the lungs also accurately recorded by a small spirometer attached to the cabinet. From the relationship of the volume change to the pressure change the volume of air in the lungs can



## LUNG VOLUME

TABLE II  
A comparison of methods for the measurement of the residual or functional residual air

Author	Method	Type of subject	Number of subjects	Number of observations	Maximum	Minimum	Average deviation from individual mean	Maximum deviation from individual mean
							cc	cc
Schenck (1894)	Pneumotometric	Normals	1	66	2065	952	±93	726
Bass (1925)	As above	Normals	6	47	1940	930	±80	347
Bohr (1907)	H <sub>2</sub> dilution of residual air	Normals	5	19	2460	710	±174	190
	As above	Normals	11	22	3650	700	±34	89
	As above	Tuberculous	8	18	2320	757	?	184
Tobiesen (1911)	O <sub>2</sub> dilution of residual air	Cardiac	5	31	1481	2008	±83	196
Peters and Barr (1920)	As above	Normals	1	15	2363	1530	±40	153
Campbell and Hill (1931)	As above	Normals	10	27	3240	960	±72	240
This paper	H <sub>2</sub> dilution of functional residual air	Normals	9	21	3697	3310	±7	10
Binger and Brow (1924)	As above	Normals and tuberculous	2 sizes	10	5120	4931	±107	280
This paper	O <sub>2</sub> dilution of functional residual air	Model lung	14	40	2710	2292	±93	170
This paper		Cardio respiratory Normals	3	25				

be calculated. Modifications of this method have been described by Neupaur (1879) Kochs (1884) Schenck (1894 (2), 1895 (2)), Bass (1925) and Wolf (1928 (3)), which give figures for the residual air ranging from 400 cc to 19,800 cc. Bass and Schenck are the only authors who give the data necessary for an estimate of how accurately observations can be duplicated on one individual (Table II). Bass himself does not discuss the accuracy of the method from this point of view and does not deny the discarding of results which show any marked deviation from the mean. From the context of his paper and results given, we suspect this to be the case.

The fallacies and drawbacks of the method are obvious. As Schenck (1894) has shown, and as we would expect, the gases in the gastro-intestinal tract are measured with the residual air. Also, the slightest leak around the mouthpiece, while the subject is making the expiratory effort, would not be shown on the tracings and yet would completely vitiate the results. The apparatus is cumbersome and its management full of technical difficulties, while more cooperation is demanded of the subject than can be given by even the most healthy individuals (Kochs (1884)).

## II GAS DILUTION WITH FORCED BREATHING

### (a) *Hydrogen Dilution*

Originally described by Davy (1800) and since modified on minor points of technique by Gréhan (1887), Berenstein (1891), Bohr (1907), Hasselbach (1908 (2) and 1912), Rubow (1908), Morawitz and Siebeck (1909), Bittorf and Forschbach (1910), Siebeck (1910 and 1912), Bruns (1910) and Tobiesen (1911), this method is based on the dilution of a known volume of hydrogen by the residual air in the lungs. After a forced expiration to expel the reserve air the subject takes from 5 to 7 deep and rapid breaths from a bag or spirometer containing a known volume of hydrogen. A homogeneous mixture of gases is then assumed to be present throughout the bag or spirometer and the lungs. A sample of this mixture is analysed to find the degree to which the hydrogen has been diluted. From the degree of dilution the volume of residual air can be calculated.

### (b) *Oxygen dilution*

Exactly the same as the hydrogen dilution method in principle, the residual air is calculated from the degree to which the nitrogen in the alveolar air is diluted by a known volume of oxygen. This method was originally developed by Durig (1903) and since by Lundsgaard and Van Slyke (1918), Peters and Barr (1920), Lundsgaard and Schierbeck (1922 and 1923), Lundsgaard (1923) and Campbell and Hill (1931).

In only four of the articles referred to is there sufficient data to form any estimate of the accuracy of either of these methods. With the exception of that by Peters and Barr, which will be discussed later, a

considerable range of variation in duplicate analyses on the same individual is shown amounting to an average deviation from the mean of from 80 cc to 174 cc and a maximum deviation from the mean of from 184 cc to 325 cc (See Table II) Using the oxygen dilution method as described by Lundsgaard and Van Slyke (1918) we have performed a series of 15 estimations of the residual air on one subject The results (Table II) show a range of from 2363 cc to 2008 cc with an average deviation from the mean of 83 cc and this in spite of the fact that the subject was an intelligent, healthy technician, long accustomed to vital capacity measurements and the Haldane Priestley method of sampling alveolar air The volume of  $O_2$  was measured to  $\pm 2$  cc and the  $O_2$  analyses done in duplicate with an accuracy of  $\pm 0.5$  per cent, a combination of sufficient accuracy to measure the volume of a model lung to within  $\pm 10$  cc (Table II)

On analysing various points of technique in the method and some of the assumptions made, it becomes obvious that a considerable range of error is to be expected in either of these two gas dilution methods Considering the wide use that has been made of these methods and their comparatively recent popularity, it might be well to discuss our criticisms in detail

(a) *True but fortuitous variations in the residual air* The inconstancy of the reserve air has already been described and it has been shown that even under carefully controlled conditions variations in this quantity may reflect changes in the residual air which are fortuitous in nature Indeed we think it probable that this inconstancy of the reserve air is mainly responsible for the variations found in the residual air of normal individuals, as measured by this method A comparison of Tables I and II certainly shows a very similar deviation from the mean

(b) *Mixing of gases by forced breathing* Owing to the popularity of gas absorption methods in the estimation of the circulation rate, the problem of gas mixing in the lungs has received considerable attention Without going fully into the controversy it may be stated that the consensus of opinion inclines to the view that in the healthy subject 5 breaths of 2 liters depth or more from a bag containing from 2 to 3 liters of a foreign gas are sufficient to ensure homogeneity throughout the lung-bag system (Krogh and Lindhard (1917), Lundsgaard (1923), Grollman and Marshall (1928), etc) In any pathological condition, however, where there is an impairment of alveolar ventilation or reduction of vital capacity, the evidence is entirely against complete mixing of gases or any homogeneity throughout the lung-bag system (Siebeck (1910, 1911 (2), and 1912), Bruns (1910), Weiss (1928), Anthony (1930)) In spite of these observations, both these  $H_2$  and  $O_2$  dilution methods for the measurement of residual air have been used on patients with all types of pulmonary impairment, in some of which the vital capacity was admit-

tedly below a liter. One such is the publication by Peters and Barr, whose duplicate analyses are summarised in Table II. All the criticisms we have to make regarding these gas dilution methods apply both to the methods and to the patients they used. It must be added that they make no statement as to the accuracy of the method and on that score may have discarded results showing any marked deviation from the mean, in which case our tabulation of their results in Table II is meaningless. In any case it is yet to be shown that measurements of this type on patients with such a low vital capacity have any real relationship to the residual air.

(c) *Inconstancy of percentage of nitrogen in alveolar air*. In the oxygen dilution method it is necessary either to measure or to assume some constant for the percentage of nitrogen in alveolar air since it is this that is being diluted with the  $O_2$  (even the respiratory dead space will contain alveolar air after expulsion of the reserve air). Various constants have been assumed for this value but actually the alveolar nitrogen shows considerable variation among different groups of patients and in individuals of the same group. In fifty-three observations on four normal individuals we have found an average of 80.28 per cent  $N_2$  in the alveolar air, in thirty-eight observations on eighteen cases with cardiorespiratory disease an average of 79.98 per cent  $N_2$ , and in fourteen observations on six cases with emphysema an average of 81.21 per cent  $N_2$ . Even these average values show considerable variations between the different groups but when we take the extremes we find a maximum of 82.33 per cent  $N_2$  in the alveolar air of a case of emphysema with extreme cyanosis and a minimum of 79.42 per cent  $N_2$  in a case of congenital stenosis of the pulmonary artery. In both these cases the  $pCO_2$  as calculated from analysis of the arterial blood closely agreed with that found in the alveolar air, so we can accept these extreme variations as being real and not due to faulty sampling.

(d) *Gas absorbed by blood*. It is known that hyperventilation produces very definite changes on the circulatory system and on haemorespiratory exchange (Marshall and Grollman (1928), Schneider (1930), Herxheimer and Kost (1931)). Any change in the circulation rate through the lungs must obviously be associated with changes in  $O_2$  consumption and also in the amount of a foreign gas which will enter into solution in the blood. To estimate the significance of these factors we have performed some experiments on a subject on whom we had made 15 determinations of the residual air by the  $O_2$  dilution method of Lundsgaard and Van Slyke (Table II). We found that during a 20 second period of hyperventilation, with 5 breaths of between 3.3 and 3.6 liters, the circulation rate ranged from 6.5 to 9.8 liters a minute, with an average of 7.8 liters in 4 observations, representing an increase of 90 per cent over his resting circulation rate. (Details of the method used have not yet been pub-

lished) With this was found an increase of from 59 per cent to 98 per cent in the oxygen consumption with an average of 73 per cent The average basal oxygen consumption was 270 cc per minute and from these figures we can calculate that the respiratory gymnastics associated with this method of measuring the residual air was accompanied by an oxygen consumption of approximately 160 cc in 20 seconds, the actual amount varying somewhat within the degree of hyperventilation If we add to this 10 cc as representing the amount of  $O_2$  taken up in physical solution at  $\frac{1}{2}$  an atmosphere pressure of  $O_2$ , we have a total of 170 cc of  $O_2$  which has passed into the blood during 20 seconds of hyperventilation If an equal amount of  $CO_2$  be excreted during this period, no error in the estimation of lung volume will result Unfortunately this is not the case The percentage of oxygen in the bag is sufficient to ensure complete saturation of the blood but since the percentage of carbon dioxide in the inspired air is continuously rising, some impairment of  $CO_2$  excretion must occur which would tend to lower the respiratory quotient The hyperaeration of the alveoli on the other hand would tend to raise the respiratory quotient The exact balance of these two factors is difficult to estimate when breathing high oxygen mixtures When room air is rebreathed under the same conditions we have found the R Q to average 0.92 on 5 estimations and we can only say that with high oxygen mixtures it must be considerably lower Even an R Q of 0.8 would lead to an error of 34 cc in the estimated lung volume under the circumstances described

With the  $H_2$  dilution method the error involved by gas absorption is small but is still present If we assume that 2000 cc of blood passes through the lungs during the period of hyperventilation approximately 15 cc of  $H_2$  will be absorbed at a pressure of  $\frac{1}{2}$  an atmosphere representing an error of less than 20 cc, with a final dilution to 50 per cent  $H_2$

(e) *Nitrogen excretion from the blood* In the  $H_2$  dilution method, the  $N_2$  excreted is of no moment but in the  $O_2$  method, where dilution of the alveolar air is the basis of calculation, all  $N_2$  excreted will be measured as if it were originally present in the alveolar air From the figures of Hill, Long and Lupton (1924), and of Campbell and Hill (1931), the  $N_2$  excreted in 20 seconds with hyperventilation and  $\frac{1}{2}$  an atmosphere of  $O_2$  would only amount to some 10 or 12 cc, which would result in an error of less than 20 cc in the residual air estimation

### III GAS DILUTION WITHOUT FORCED BREATHING

#### (a) *Hydrogen dilution*

A very definite advance in the technique of lung volume measurement was made when Van Slyke and Binger (1923) described their method for the estimation of the residual air, or functional residual air, without forced breathing, and it is remarkable how few have availed themselves

of this excellent method or its modification by Binger and Brow (1924). The method is based on the mixing of a known volume of hydrogen with the nitrogen in the lungs, mixing being accomplished by quiet respirations from a spirometer for from 5 to 7 minutes. The volume of air in the lungs at the moment the patient is switched to the spirometer is calculated from the volume of hydrogen originally added to the spirometer and the ratio of the percentage of hydrogen after mixing has been accomplished. Those who have worked with the method have found it entirely satisfactory (Binger (1923), Binger and Brow (1924), Meakins and Christie (1929), and Anthony (1930)), but unfortunately only one of these papers contains the data necessary for an estimate of the accuracy of the method when applied to the human subject. Meakins and Christie have used this method on a limited number of subjects with satisfactory but less impressive results than those obtained by Binger and Brow (Table II). It must be stated, however, that our own figures were obtained with the use of a Haldane gas analysis machine, using the micro method, and part at least of the variation found by us may have been caused by errors in analysis. Anthony (1930), although no protocols are published, finds with this method a maximum variation of 100 cc. and an average variation from the mean of 50 cc.

From a technical point of view the only criticisms of this method we can offer are the errors from absorption of hydrogen and excretion of nitrogen by the blood, but with similar technique it seems improbable that these factors should vary greatly from time to time in the same subject. With a final mixture of 30 per cent  $N_2$  it can be estimated from the figures of Hill, Long and Lupton (1924) and Campbell and Hill (1931) that at least 65 cc. of  $N_2$  are excreted into the lungs in 5 minutes. No figures for the absorption of hydrogen are available but if we take the absorption coefficient of hydrogen as 0.01644 and the final percentage as 30, and assume gaseous equilibrium to have been established between the alveolar air and 10 liters of body fluids (an amount which is probably too low), it can be calculated that approximately 50 cc. of hydrogen have been absorbed from the lungs. If 1500 cc. of  $H_2$  were originally added to the spirometer and 1500 cc. of  $N_2$  were present in the alveolar air, this haemorespiratory exchange would result in a measured  $\frac{N_2}{H_2}$  ratio of 1.07 instead of the true ratio of 1.00. The resultant error in the lung volume estimation would amount to 105 cc., a figure very close to that assumed by Anthony (1930) but a figure which, in our opinion, is minimal, 150 cc. or 200 cc. being equally probable. Again a figure for the percentage of nitrogen in the alveolar air has to be assumed and a certain error will result. In the extreme case of emphysema quoted above with an alveolar  $N_2$  of 82.33 per cent this error would amount to some 60 cc. even if the residual air were normal and not increased, as was the case. Mixing of gases through out the lung spirometer system, on the other hand, has been shown to be

complete, both in normals and in those with cardiovascular disease (Van Slyke and Binger (1923))

The main drawback of this method is one that appeals perhaps to the less scientific and more imaginative members of the medical profession. The routine use of an explosive mixture in respiratory experiments is considered by some to be unsafe and to my knowledge has frightened several investigators from the method. The possibility of arsenical poisoning must also be excluded. In a laboratory with careful workers we do not see that there is any danger, but have also found it difficult to convince both the laity and some of the medical profession. It was mainly for this reason, but also in the hope that we might be able to simplify the technique of lung volume measurement, that we have developed a method which requires little but what is available in every well-equipped hospital laboratory.

#### *(b) Oxygen dilution without forced breathing*

The principle underlying the method is again the dilution of the nitrogen in the lungs with a known volume of oxygen, but complete mixing is ensured by adopting the technique of Van Slyke and Binger. At the same time some of the drawbacks of their method are avoided.

#### DETAILS OF THE APPARATUS

The spirometer is of the standard type as commonly used in the estimation of the basal metabolic rate, with a capacity of from 6.5 to 8 liters (preferably the latter) and equipped with the usual ink recording attachment and thermometer. The counterpoise is adjusted so that balance is perfect with the bell at half capacity. The soda lime scrubber should be removed from the body of the spirometer and this space filled with solid paraffin to form a solid central core pierced by the inspiratory and expiratory tubes. The spirometer dead space is then reduced to a minimum, as when the bell is lowered its interior is almost completely occupied by the solid centre core, the thermometer projecting into either the inspiratory or expiratory tube. The inspiratory and expiratory valves are placed outside the spirometer and as near to the mouthpiece as is convenient. Between each valve and the spirometer is placed a 3-way tap so that in the one position the subject breathes to and from the room, and in the other, to and from the spirometer. A soda lime scrubber is inserted on the expiratory side of the circuit between the 3-way tap and the spirometer. Between the scrubber and the spirometer is the usual side tap for the collection of samples. With the bell fully lowered the volume of air in the whole circuit, from mouthpiece to spirometer and spirometer to mouthpiece, should not exceed 3,000 cc or at the most 3,500 cc (see measurement of spirometer dead space) and this quantity should be kept absolutely constant. All tubing connecting the mouthpiece with the spirometer should be of such rigidity and so arranged that the volume of its lumen will remain constant. We have found short lengths of glass tubing connected with rubber tubing very satisfactory. The volume of soda lime in the scrubber is kept constant by weighing. It is also important that there should be no variation of the level of water in the water seal. This is easily avoided by the use of a small syphon manometer so placed that it will not interfere with movements of the spirometer bell.

Gas analysis may be accomplished by any of the many methods capable of analysing up to 50 per cent oxygen mixtures with an accuracy of  $\pm 0.1$  per cent or less. A Haldane gas analysis machine graduated to the 5 cc. mark has been found satisfactory although the absorption of oxygen is somewhat tedious. We have collected the samples over mercury in a Haldane sampling tube, and analyses have always been done in duplicate.

### *Measurement of spirometer dead space*

With such a complicated system it is impossible to obtain any accurate calculation of the volume of air in the spirometer dead space (i.e., the volume of air in the spirometer and tubing with the bell empty) by simple linear measurements. Much more accurate is its measurement by gas dilution, which serves the double purpose of giving a figure for this quantity, and also of standardizing the accuracy of the gas analysis and other points of technique. We have found the following technique for the measurement of the dead space simple, and surprisingly accurate. A rubber bag of from 2 to 6 liters capacity and with an air opening at both ends (such as is commonly used in the administration of gas anesthetics) is equipped with two glass stopcocks, one at either opening. One of these taps (Tap A) is connected to the mouthpiece by a short piece of rubber tubing, so that when open, the bag is in direct communication with the spirometer. The other tap (Tap B) leaves the bag open or closed to the atmosphere. With Tap A closed and the spirometer empty (having been previously thoroughly flushed through with room air), the 3 way taps are so turned that the airways are open from mouthpiece to spirometer. The rubber bag is completely evacuated by suction and Tap B closed. A carefully measured volume of oxygen is then run into the spirometer, Tap A opened, and by using the spirometer bell as a pump the gases in the circuit are thoroughly mixed. A sample is then taken from the spirometer and the oxygen percentage determined. The volume of the spirometer dead space is then calculated as follows

$$x \frac{79.1}{100} = (x + a) \frac{y}{100}$$

or

$$x = \frac{ay}{79.1 - y}, \quad (1)$$

where  $x$  = the volume of the dead space in cc.,

$y$  = the percentage of nitrogen in the sample taken after mixing is complete,

$a$  = the amount of oxygen introduced into the spirometer in cc.

Duplicate measurements show a very close agreement. Five measurements made on the spirometer circuit described above, and 5 on a standard basal metabolic rate machine, gave an average deviation from the mean of 7 cc. and a maximum deviation of 10 cc. (Table II, "Model Lung")

### *The measurement of the functional residual air*

The spirometer circuit is thoroughly flushed with room air using the bell as a pump. Our routine has been 3 series of 4 to 5 liter excursions of the bell with an interval of 2 to 3 minutes between each to allow for diffusion of oxygen from the soda lime and other parts of the circuit. The spirometer bell is then emptied and the two 3 way taps turned so that the mouthpiece leads to the



room air. A carefully measured volume of  $O_2$  is run into the spirometer (we have found it more accurate to measure this volume of oxygen from a tracing taken with the pen attachment than from direct observation of the scale), three minutes being allowed for temperature equilibrium to be reached, and a thermometer reading is taken. The subject who has been lying in the dorsal decubitus with the arms by the side, shoulders resting on the bed, and one pillow supporting the head, for at least 15 minutes, is then attached to the apparatus by means of the usual rubber mouthpiece and nose-clip. He breathes room air for 2 minutes, the drum is started and the subject is then suddenly switched to the spirometer by turning both 3-way taps simultaneously, preferably at some point during the respiratory cycle near the height of inspiration. After a period of seven minutes' quiet breathing, the 3-way taps are again turned, disconnecting the patient from the spirometer. A weight is placed on the bell of the spirometer, and left for three minutes during which time any leak will show itself. The small tap for the collection of samples is opened and from 2 to 3 liters of gas allowed to escape. A sample of the air in the spirometer can then be collected for analysis. The following practical details are essential if accurate results are to be expected. If the subject is unaccustomed to breathing from a spirometer practice should be given prior to the experiment. The subject should be unaware of the purpose of the experiment, as any conscious effort to maintain an even resting respiratory level usually results in hopeless irregularities. It has been our custom to inform the subject that it is just a "breathing test," that he will feel nothing, need only breathe quietly and can go to sleep if he wants to. Leaks around the mouthpiece should be carefully avoided. It has been our custom to moisten the mouthpiece with water or dilute glycerin and observe the mouth throughout the experiment. Resistance to respiration should be minimal and any change in resistance, such as is easily produced by sticking of the rubber valves, should be carefully avoided. The influence of resistance on the resting respiratory level has already been mentioned. In one of our normal subjects we have found that a slight increase in the expiratory resistance, although insufficient to affect the respiratory rhythm, raised the functional residual air from an average of 2425 cc in 15 estimations to an average of 2750 cc in 5 estimations. For these reasons the respiratory valves should be examined daily for any stickiness of the valve flaps. The efficiency of the soda lime scrubber should be checked at regular intervals by analysing the spirometer air for  $CO_2$ , if this rises above 0.2 per cent the soda lime should be changed.

#### CALCULATION

The actual volume measured by this method is obviously the amount of air in the lungs at the moment the patient is switched to the spirometer. The nitrogen in the lungs at this moment is diluted by a known volume of oxygen, and from the degree of dilution the lung volume can be calculated from the equation,

$$\lambda \frac{v}{100} + d \frac{79.1}{100} = (\lambda + d + a - b) \frac{y}{100},$$

where  $\lambda$  = the lung volume in cc,

$v$  = percentage of  $N_2$  in lungs at beginning of experiment,

$y$  = percentage of  $N_2$  in the circuit at end of experiment,

$a$  = oxygen in spirometer at beginning of experiment in cc,

$b$  = oxygen absorbed during experiment in cc,

$d$  = dead space in spirometer circuit in cc

Unfortunately for the utility of this equation the value of  $v$  has been shown to be by no means a constant and is not measured in each subject. Also, there is no justification for the assumption that at the end of the experiment there is any homogeneity throughout the lung spirometer system. Indeed it is only reasonable to suppose that, at the time the sample is taken, the nitrogen in the inspired air (i.e., the nitrogen in the spirometer) is of lower percentage than the nitrogen in the alveoli, since more oxygen is being absorbed than  $\text{CO}_2$  excreted, and therefore a process of  $\text{N}_2$  concentration is continuously taking place in the lungs. However, the rise in the percentage of  $\text{N}_2$ , which takes place when air is inhaled from the spirometer, must be equivalent to the rise which takes place when air is inhaled from the room, since in both the lungs are performing the same process of  $\text{N}_2$  concentration. Applying these theoretical considerations to our technique of lung volume measurement, it is clear that if we use  $v$  (the alveolar  $\text{N}_2$  percentage at the beginning of the experiment) to represent the amount of  $\text{N}_2$  being diluted, the sample  $y$  (the percentage of  $\text{N}_2$  in the circuit after mixing is complete) must also be taken from the alveolar air. But since the difference between  $v$  and the room air nitrogen is equivalent to the difference between  $y$  taken from the alveoli and  $y$  taken from the spirometer, we are perfectly justified in substituting the room air nitrogen (79.1 per cent) for  $v$ , if the sample from which  $y$  is estimated is taken from the spirometer.

Our equation will then simplify itself to

$$x \frac{79.1}{100} + d \frac{79.1}{100} = (x + d + a - b) \frac{y}{100}$$

or

$$x = \frac{y(a - b)}{79.1 - y} - d, \quad (2)$$

the meaning of  $x$ ,  $a$ ,  $b$  and  $d$  remaining unchanged but  $y$  signifying the percentage of nitrogen in the spirometer at the end of the experiment. In this way those errors accruing from the inconstancy of the alveolar nitrogen are made to compensate for the lack of homogeneity throughout the lung spirometer system after so-called mixing has been completed. Actually it can be shown that compensation for these errors is not quite complete. If room air be inspired and 7 cc. per cent of  $\text{O}_2$  are absorbed, and 6 cc. per cent of  $\text{CO}_2$  excreted, then the percentage of  $\text{N}_2$  will rise from 79.1 in the inspired air to 79.9 in the expired air, a rise of 0.8 per cent. If the oxygen of the inspired air be raised to 45 per cent, then with the same metabolic factors the percentage of  $\text{N}_2$  will be raised from 55 to 55.6, a rise of 0.6 per cent. Since the respiratory dead space is a constant factor throughout, these differences between the inspired and expired nitrogen are exactly proportional to the differences between inspired and alveolar nitrogen. As shown, this difference does vary somewhat with the percentage of nitrogen inspired, the change when breathing 55 per cent  $\text{N}_2$  amounting to only 75 per cent of that when breathing room air. This discrepancy has to be disregarded in our calculations, but the error involved is a small one, amounting to some 10 to 20 cc. in the functional residual air of an average subject.

As already stated, we have found that a more accurate measurement of the amount of oxygen added to the spirometer is obtained by graphic registration than by a direct reading on the scale. The oxygen absorbed is measured in the usual way by a line drawn through the resting respiratory level. Difficulties in the measurement of this quantity are discussed below.

The actual volume measured by the above technique and by the use of

formula (2) is the amount of air in the lungs at the moment the subject was switched from breathing room air to the spirometer. The height of this point above the resting respiratory level is measured from the respiratory tracing. To obtain the value of the functional residual air, this amount is subtracted from  $x$ . The quantity so obtained represents the volume of the residual air at the temperature of the spirometer air. This must be corrected to 37° C saturated with water vapor. It has been the custom of some to correct also for the barometric pressure but it has yet to be shown that the barometric pressure has any influence on lung volume.

To allow for nitrogen excretion we have estimated from the figures of Hill, Long and Lupton (1924) and Campbell and Hill (1931) that approximately 65 cc of nitrogen will be excreted in 7 minutes when breathing an atmosphere of 44 per cent oxygen. We have therefore subtracted 80 cc in all cases from the measured functional residual air. Obviously no correction need be made for oxygen passing into solution, as this will be registered as oxygen consumption.

From the functional residual air, the total capacity and residual air can be calculated by adding the complemental and subtracting the reserve air respectively. It has been our custom to measure these immediately after the lung volume estimation and with the subject in the same carefully controlled position.<sup>2</sup>

#### CRITICISM

In our opinion the main drawback to this method is the difficulty in obtaining an accurate estimation of the oxygen consumption on some subjects. However, one can always tell from a 7 minute respiratory tracing approximately how much room there is for error. Our routine has been to draw the base line, which we think represents the oxygen consumption, and if one has to admit the possibility of more than a 50 cc error in the total oxygen consumed the experiment is discarded or, if a better tracing is not procurable, the possible range of error is noted. With a final mixture of 45 per cent oxygen, an error of 50 cc in the oxygen consumption will lead to an error of 93 cc in the lung volume. The only other criterion for the discarding of an experiment has been the possibility of a leak as shown by the spirometer tracing.

Nitrogen excretion probably varies somewhat from patient to patient

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<sup>2</sup> Sendroy, Hiller and Van Slyke (1932) have recently published a method for the determination of lung volume by respiration of oxygen without forced breathing. In their method it is essential that "at the end of the period the subject brings his lungs to the same position as at the beginning." They have only used this method for the estimation of the residual air but when we consider the fluctuation of the reserve air described above it becomes evident that expert cooperation is necessary, and accurate results can hardly be expected from patients with any impairment of respiratory function. The authors do not describe any measurements of the functional residual air, and we hardly think that this would be feasible with their method. We have always found that any such voluntary control of the resting respiratory level even in trained individuals leads to marked irregularities.

but we cannot believe that with similar experimental technique this quantity can vary by more than 10 or 20 cc representing a final error of from 15 to 25 cc.

The question of mixing throughout the lung-spirometer circuit cannot be so satisfactorily approached by this method as by the method of Van Slyke and Binger (1923), since the nitrogen percentage is falling at an increasing rate throughout the experiment, and since the sample taken must represent the air in the spirometer bell. Van Slyke and Binger have conclusively shown that both in cardiacs and in normal subjects the technique we have used is sufficient to ensure mixing in 5 minutes. To make doubly sure we have extended this time to 7 minutes.

In 25 measurements of the functional residual air in 3 normal subjects we have found an average deviation from the mean of each subject of 93 cc, and a maximum deviation of 170 cc. In 40 measurements on 14 subjects with cardio-respiratory disease, ranging from advanced emphysema to spontaneous pneumothorax, we have found an average deviation from the mean of each subject of 107 cc. and a maximum deviation of 280 cc. When these results are compared with those by other methods (Table II) it must be remembered that at least in some instances in this group results showing marked deviation from the mean have been discarded. Moreover, only this method and the method of Van Slyke and Binger can be used with any justification in those cases where there is any marked impairment of vital capacity or pulmonary ventilation. In this type of case our method may or may not be as accurate as the method of Van Slyke and Binger, but in its simplicity and applicability to clinical use it certainly presents some advantages.

#### SUMMARY AND CONCLUSION

(a) A simple classification of the subdivisions of the total lung volume is proposed.

(b) The complemental, reserve and residual airs can show considerable variations which are purely fortuitous in nature, even under carefully controlled conditions on one individual. Similar variations could not be demonstrated in the functional residual air.

(c) The methods for the determination of the residual and functional residual air are reviewed, and experimental evidence given to show that most of these will give fallacious results in both normal and abnormal individuals.

(d) A simple method is described for the measurement of the functional residual air.

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# THE UREA CLEARANCE TEST IN TOXEMIAS OF PREGNANCY

## (A PRELIMINARY REPORT)

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At present there is no adequate study of the value of measuring renal function or the changes in blood chemistry in order to differentiate the toxemias of pregnancy from chronic nephritis or demonstrate the return of function of kidneys acutely damaged by severe toxemia or eclampsia. It appears from work previously done that the urea clearance test of Van Slyke and his associates offers a much more accurate method of study of renal function than other tests in common usage. For example, it has been shown that the blood urea clearance must usually fall below fifty per cent of its normal value before significant changes occur in blood creatinin, blood urea nitrogen, blood uric acid, or phenolsulphonephthalein values, and that only after the urea clearance falls below twenty per cent of normal is there usually a change in all of the above values (1, 2).

Consequently, it seemed worth while to apply the urea clearance test to a series of patients entering the Boston Lying in Hospital with a diagnosis of toxemia or nephritis and to a similar group followed in the out-patient toxemia clinic of the Hospital. It also appeared advisable to make a comparative study between data obtained from the urea clearance test and those obtained from other more common methods for studying kidney function. In this report, such comparative data have been limited to the blood nonprotein nitrogen, blood uric acid, blood urea nitrogen, one-hour water test for dilution and concentration, and the phenolsulphonephthalein test.

The patients were classified into toxemic, chronic nephritic and eclamptic groups and a normal pregnant control group. The dividing line between toxemia and chronic nephritis was frequently indefinite, but an attempt was made to include under chronic nephritis the following patients: those who had a definite history of nephritis just before becoming pregnant, those who had a high blood pressure and albuminuria in the early months of pregnancy (up to the fifth month), and those who continued with high blood pressure (over 160 mm Hg systolic) for some months after delivery. A few of the last mentioned are probably not true nephritics at the present time, but for the purpose of this report are considered thus until proved otherwise. Patients were classified as



toxemics when they had blood pressures over 140 mm systolic and showed albumin in a catheter specimen. A few of the toxemics were severe enough to be called pre-eclamptic. The toxemics were all in the latter part of pregnancy (after the seventh month). The eclamptics all had hypertension, one or more convulsions, diminished urine output, and albuminuria.

#### METHODS AND NORMAL VALUES

1 Nonprotein nitrogen was determined by the method of Folin (16). The normal limits are 25 to 40 mgm per cent, although it is normally lower in pregnancy.

2 Uric acid was determined by the method of Folin and Benedict (15). The normal limits are 2.5 to 5.0 mgm per cent.

3 Blood urea nitrogen was obtained by the gasometric method of Van Slyke (3). Although the upper limit of normal is 23 mgm per cent, it is lower in pregnancy. Folin gives 5 to 9 mgm per cent as the normal range in pregnancy. We have taken 12.5 mgm per cent as the upper limit of the blood urea nitrogen in normal pregnancy.

4 The one-hour test was carried out as follows: breakfast is omitted and 1000 cc of water is given at 8:00 A.M., specimens are collected hourly until lunch, which is dry, and then at 4 P.M. and 6 P.M. Normally, a patient should dilute to 1:003 or less, concentrate to 1:024 or more and excrete the liter of water in the first four hours. The normal pregnant patients and a few patients in the other groups were given concentration tests, i.e. four urine samples were collected hourly in the morning after abstaining from fluids for twelve hours. Normally, a patient should concentrate to 1:025.

5 The phenolsulphonephthalein test was done as follows: after the patient has voided, the dye is given intramuscularly. A glass of water is then given and repeated in one hour. Two hours and ten minutes after the injection, the urine is collected, made alkaline and compared with standards (17). Normally, at least 55 per cent of the dye is excreted.

6 The urea clearance test was done as follows: the patient is given nothing to eat or drink after midnight before the test. Breakfast is omitted. At 7:45 A.M. she is given 200 cc of water. At 8:00 A.M. the patient voids and is immediately catheterized for residual urine. If the residual urine is over 30 cc she is catheterized two hours after the first catheterization, otherwise she is allowed to void at 10 A.M. A blood sample is taken one hour after the first voiding. The urea in blood and urine was determined by the method of Van Slyke (3). The patient is kept in bed during the test. The height and weight is obtained. The clearance may be expressed in "the number of cc of blood cleared of urea per minute," or in per cent of normal clearance, which we have used because it is easier thus to compare the standard with the maximum clearance. For derivation of formulae, see papers of Van Slyke and associates (4, 5, 7, 8). Correction for body surface was applied (6). The lower limit of normal is about 80 per cent.

#### RESULTS OF BLOOD CHEMICAL STUDIES AND FUNCTIONAL TESTS

In Tables I, II, III and IV, we have summarized the results of the entire study. In instances where more than one test has been done, we have simply indicated the range of values without regard to sequence. Inasmuch as we are interested primarily in the comparison between the

urea clearance values and the values found in other tests, we have separated, wherever possible, the data in these tables into two sub-groups, one containing those cases in which the urea clearance was normal and the other, those cases in which the urea clearance was abnormal

TABLE 1  
*Toxemia group*

21 cases	Non protein nitrogen	Blood urea nitrogen	Uric acid	One-hour test		Phenol sulphone-phthalein	Urea clearance
				Low specific gravity	High specific gravity		
	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.			per cent	per cent of normal
Sub-Group I (Normal Urea Clearance)							
II		4.8					178
III	26-29	6.2	3.5-3.4				157
IV	28-31	6.7	5.1	1.002	1.010	40	108
V	20-25	4.6	2.7-3.7	1.007	1.018		144
VIII	30-39	10.6	3.1-4.0	1.003-05	1.012-15	50	90-109
IX	30-31	4.5	3.0-5.2	1.007	1.018		130
X	22-34	5.6-10.3	3.3-3.4	1.002	1.026	40	80-123
XI	22	4.6	3.9	1.008	1.021		107
XII	25	3.6	8.3				116
XVI	25	4.8-5.2	3.8	1.002	1.025	75	112-125
XVIII	33-40	12.2	3.7-5.7	1.005	1.022		161
XX	24-43	4.1-5.8	3.3-6.2				101-160
XXI	27-30	2.8	3.4-3.7	1.008	1.026	40	173
Sub-Group II (Low Urea Clearance)							
I		7.9					79
VI	31-41	7.9-12	3.9-4.6	1.006-07	1.010-26		36-93
VII	33-41	8.0-8.6	3.0-6.1	1.009	1.016	60	58-86
XIII	27-28	6.7-8.6	3.8-4.4	1.003	1.025		60-97
XIV	30-39	6.3-9.2	4.0-4.1	1.002-05	1.010-13		66-99
XV	27-31	11.9	4.2-5.8	1.002	1.020	55	66
XVII	35-40	6.2	3.3-3.7	1.005	1.022	50	79
XIX	24-31	5.6	3.5	1.005	1.016		70-90
Averages for entire group							
	30	6.9	4.3	1.005	1.019	51	111
Per cent abnormal findings							
	0	0	11		82	63	14

*Toxemia group*

In the sub group in which the urea clearance tests are within normal limits, abnormalities appear in the other tests, particularly in the uric acid and one-hour test. In like manner, in the sub-group in which the

urea clearance values are low, normal values appear in the other tests, including the one-hour test. In other words, the urea clearance values do not agree with the values for the other tests, nor do the findings among the other tests agree with each other. It is apparent, therefore, that the values for the urea clearance test are independent of values found among

TABLE II  
*Chronic nephritic group*

17 cases	Non-protein nitrogen	Blood urea nitrogen	Uric acid	One-hour test		Phenol-sulphone-phthalein	Urea clearance
				Low specific gravity	High specific gravity		
	<i>mgm per 100 cc</i>	<i>mgm per 100 cc</i>	<i>mgm per 100 cc</i>			<i>per cent</i>	<i>per cent of normal</i>
Sub-Group I (Normal Urea Clearance)							
XXII	20	6.2	3.0				84
XXXI	29	3.8	4.2				84
XXXVII	26	7.2	6.5	1.006	1.020	50-55	111
Sub-Group II (Low Urea Clearance)							
XXIII	30-37	5.1-10.8	3.1-4.6	1.003-07	1.011-18	20-80	66-76
XXIV	29-31	7.2-9.6	4.6-5.4	1.005	1.013-20		51-72
XXV	29-57	12.7	6.5-8.0				26
XXVI	35-41	4.6-6.6	2.9-5.0	1.008	1.017	50	54-85
XXVII	21	8.8	2.8	1.003	1.023		31
XXVIII	39-46	6.7-10.1	4.6-5.4	1.002-05	1.013-15	15-60	57-75
XXIX	24-28	8.5	4.0-4.4	1.005	1.020-30	40	59
XXX	38-39	8.6-22.0	4.2-4.7	1.010	1.020	40	32-50
XXXII		8.5		1.004	1.017		71
XXXIII	27-29	9.0-9.2	3.6-5.0	1.002	1.014-24		79-81
XXXIV	28-38	9.4-12.1	4.1-5.2	1.006-20	1.022-24	45	76-121
XXXV	22-30	16.9		1.003-07	1.020-25		41
XXXVI	28-39	14.2-15.1	4.7-5.7	1.006	1.034		41-78
XXXVIII	37-39	13.8	4.9-5.1	1.000	1.017		28
Averages for entire group							
Per cent abnormal findings	32	9.8	4.6	1.005	1.020	45	64
	12	29	20	79		100	67

the other tests employed. It also appears that in the toxemia group a large percentage of the urea clearances (87 per cent) are within normal limits. Furthermore, we have data, not included in the table, on a group of sixteen patients with a previous history of toxemia, eleven of whom are now pregnant again. In this group we have found normal urea clearances throughout.

*Chronic nephritic group*

In the sub group of normal urea clearances, we have recorded only three cases. It is possible, however, that the urea clearance values in Cases XXXIII and XXXIV might well be considered within normal limits. It will be noted that the highest values for the urea clearance in the chronic nephritic group are to be found in Case XXXIV and Case XXXVII. It so happens that there is some question concerning the clinical diagnosis of nephritis in these two cases, discussion of which will be found later in the text. In the remaining two cases in Sub group I (XXII and XXXI), the urea clearance values are only slightly above

TABLE III  
*Eclamptic group*

5 cases	Non protein nitrogen	Blood urea nitrogen	Uric acid	One-hour test		Phenol-sulphone-phthalein	Urea clearance
				Low specific gravity	High specific gravity		
	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.			per cent	per cent of normal
(Low Urea Clearance) *							
XXXIX	32-42	4.9-8.5	3.0-6.3				55-80
XL	36	15.9	5.8				8
XLI	26-43	5.3-11.5	3.2-7.8	1.009	1.022	70	70-82
XLII	32-33	11.1-19.0	4.2-5.8	1.005	1.020	40	16-82
XLIII	37	4.5-9.8	4.2-4.6	1.004	1.019	80	57-150
Averages for entire group	36	10.7	5.2	1.006	1.020	63	61
Per cent abnormal findings	0	40	60	100		67	60

\* All eclamptics had at least one abnormal urea clearance

the lower limit of normal. Apart from the above five cases, the urea clearance findings for the group stand out as definitely abnormal. Furthermore, a comparison of the urea clearance values with values for the other tests reveals the same lack of agreement as was noted in the toxemia group. In the clinical material under consideration, we are dealing with cases in which nephritis, if present at all, is present to only a slight or moderate degree. In other words, we are seeking a method of detecting early kidney damage. As a result of our studies, we are forced to conclude that tests other than the urea clearance test do not give information sufficiently reliable or consistent to be of value in the problem of differential diagnosis here presented. It is true that our clinical group is a small one, but it is also true that our conclusions are in agreement

with the findings at the Boston Lying-in Hospital where the above mentioned tests have been done as a routine for a period of years. Whether or not the urea clearance findings are of value as an aid in the ultimate diagnosis, we can not say until the clinical material has been observed over a period of time. We can say this much, however, that in the group here presented, the urea clearance findings compare favorably with clinical impressions.

TABLE IV  
*Normal pregnancy group*

5 cases	Non-protein nitrogen	Blood urea nitrogen	Uric acid	One-hour test		Phenol-sulphone-phthalein	Urea clearance
				Low specific gravity	High specific gravity		
	<i>mgm per 100 cc.</i>	<i>mgm per 100 cc</i>	<i>mgm per 100 cc</i>			<i>per cent</i>	<i>per cent of normal</i>
(Normal Urea Clearance) *							
XLIV		8.0			1.032		93
XLV		7.0			1.019		83-97
XLVI		4.8-6.8			1.021		136-154
XLVII		7.1-9.8			1.032		129-161
XLVIII		5.0			1.024		115-153
Averages for entire group	6.9				1.026		127
Per cent abnormal findings	0				40		0

\* There were no abnormal urea clearances in the normal pregnancy group.

#### *Eclamptic group*

In this small group of eclamptics, each patient had at least one abnormal uric acid determination and one abnormal urea clearance. A more detailed discussion of the individual cases follows.

#### *Normal pregnancy group*

Three of the patients in this group showed a lack of ability to concentrate urine to 1.025 whereas none of them showed any abnormalities in the urea clearance test.

#### GENERAL DISCUSSION OF THE UREA CLEARANCE FINDINGS

In our study there were one hundred three urea clearance tests done on sixty-four patients. In the toxemia group, the average urea clearance was 111 per cent, which is well above normal. In eight cases the urea clearance was slightly low during the acute stage, the five that could be repeated became normal. In general, our results in the toxemia group

differ from those of Spalding, Shevky and Addis (11), who found low Addis ratios in toxemia. Their cases, however, included eclamptics and chronic nephritics. Stander's (12) results are similar to ours. In patients with a previous toxemia, the average of the tests done on those pregnant again without toxemia was 126 per cent. One in the series was low but was normal a few weeks later. A non pregnant group with a history of previous toxemia all were normal (average 91 per cent).

The urea clearance in the chronic nephritic group averaged 64 per cent (about one half the average normal pregnant value). One patient (XXXIV) had normal urea clearances the first two times (121 and 104 per cent). She was a thirty-eight year old multipara who had a high blood pressure and a cloud of albumin in the fifth month, with a history of similar trouble in her last two pregnancies. Her third urea clearance was slightly low (76 per cent). After a hysterectomy, she had continued to have hypertension, but the albumin cleared up in a week. Two other patients (XXXIII and XXXVII) had hypertension, 160 to 170 systolic, but no albumin since delivery,—one was two to three months postpartum, the other about a year. The urea clearances of the first averaged 79 per cent (borderline) while that of the second was 111 per cent. These patients had hypertension but were not definite nephritics. Repeated tests on them would be interesting. We do not believe it fair to make a diagnosis of nephritis on the basis of an increased blood pressure alone.

The urea clearance in the eclamptic group averaged 61 per cent. The group is small and the results scattered because of the marked differences in the general and renal condition of the patient. The first patient was a very sick eclamptic who ran a high blood pressure (190 systolic, 85 diastolic) for thirteen days and then had a drop to 130 systolic, 100 diastolic and had albumin for eleven days after delivery. Her last test was 55 per cent, which was lower than her earlier tests. We have not seen her since her discharge and it is too early to say much about persistent renal impairment. The next case with 8 per cent was almost anuric during the test and died the next day. The fourth case in the eclampsia group showed a steady rise in urea clearance—the first, 16 per cent, was during her sickest period when urine was scanty, the second, 48 per cent, was done two weeks later, and the third, 82 per cent, about two months after leaving the hospital when she felt perfectly well, had no elevation of blood pressure or albumin in the urine. This rise in urea clearance with the patient's recovery is very striking. We hope to extend our observations to other cases of this sort. Apparently in eclampsia, the urea clearance is low not only because of the decreased volume output, but also because of a low urea concentration factor. In our small series, there was either a striking return to normal clearance, or there was a persistently low clearance value as in the first eclamptic. Possibly the latter will go on to chronic nephritis as is known to occur so

often after eclampsia (13) Further application of this test to the eclamptic group is desirable and may give very important information

In the normal pregnancy group the average of the urea clearances is 127 per cent, which is higher than the average non-pregnant normal Spalding, Shevky and Addis also obtained high results in the Addis ratio in normal pregnancy (11) Possibly this explains the low blood urea nitrogen found in normal pregnancy (10) The normals were first obtained at about the fifth month of pregnancy and are being followed throughout pregnancy

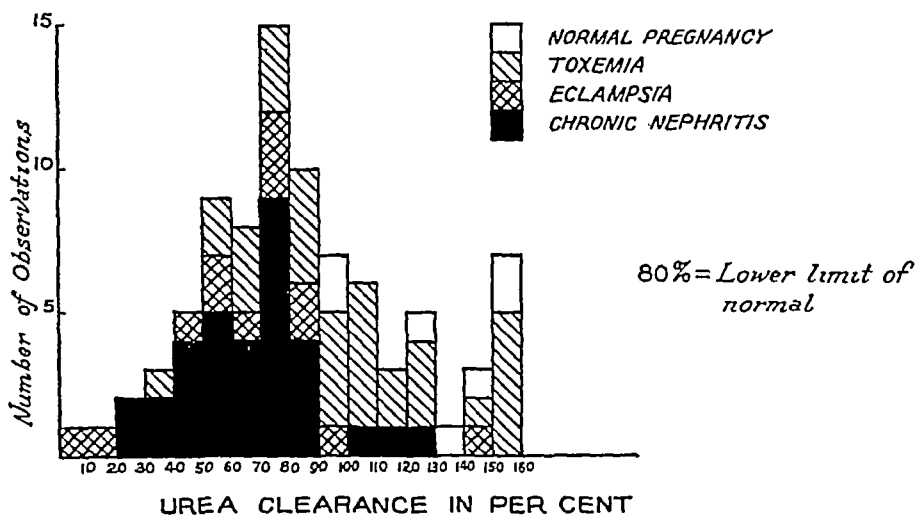


FIG 1 DISTRIBUTION OF UREA CLEARANCE TESTS IN NORMAL PREGNANCY, TOXEMIA, ECLAMPSIA AND CHRONIC NEPHRITIS

Figure 1 illustrates the distribution of individual urea clearance tests in toxemia, eclampsia and chronic nephritis. It is noteworthy that below 75 per cent (lower limit of normal) all but six tests were in nephritics and eclamptics. Of these, four were repeated soon after and found to be normal, the other two have not returned to the clinic. There are seven normal clearances in the nephritic group on six patients, of whom three had other low tests and two were questionable nephritics impossible to diagnose at the present time. It may be seen that the preponderance of tests done in the chronic nephritic group was below 80 per cent, while that of the toxemic group was above 80 per cent. The tests done in the eclamptic group were scattered for reasons mentioned above.

Van Slyke and Cope (14) have recently described a simplified method of determining the urea clearance, which, while it is not quite as accurate as the method used above, saves time when several determinations are made at once.

We wish to acknowledge the interest and aid of Dr F C Irving, Dr Saul Berman, and Dr M V Kappius. Mr James Quinn made the routine chemical analyses.

## SUMMARY AND CONCLUSIONS

1 Blood chemistry tests, phenolsulphonephthalein and one-hour tests did not help in the differentiation between chronic nephritis and acute toxemias

2 One hundred three urea clearance tests were done on 64 patients falling in groups of normal pregnant, toxemics, eclamptics, patients who had toxemia in a previous pregnancy, and chronic nephritics

3 The urea clearance checked up well with the clinical diagnoses with only a few exceptions. It was found to be higher than the usual normal limits in normal pregnancy, normal in toxemias, decreased in the acute stage of eclampsia (with tendency to rapid return to normal in one case) and low with a high degree of consistency in chronic nephritis

4 The data suggest a correlation between the high urea clearance and the low blood urea nitrogen in normal pregnancy

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# GASTRO-INTESTINAL STUDIES I GASTRIC JUICE IN PERNICIOUS ANEMIA

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## GASTRIC JUICE IN PERNICIOUS ANEMIA

Several authors have indicated that the determination of the hydrochloric acid, in itself, is not an adequate measure of the impairment of the secretory activity of the gastric mucosa Michaelis (1) demonstrated this fact by the simultaneous determination of acid and enzymes in gastric juice from cases of low and high acidity and achylia Pollard and Bloomfield (2) stated that a low pepsin output is a more delicate index of gastric function than low acid values Davies (3) similarly reported low pepsin values in the achylia of pernicious anemia

Since the determination of the enzyme content of the gastric juice may be a valuable aid in the differential diagnosis of certain types of anemia, we are presenting the data that we have obtained in a series of pernicious anemia cases and normal controls In a later paper data will be presented on secondary anemia and some obscure types of anemia

In this series of experiments we have determined the gastric enzymes, pepsin and rennin, the free and total acid, the hydrogen ion concentration and the nitrogen, chloride, and phosphorus content, in order to determine as fully as possible the extent of the dysfunction of the gastric glands in the achylia gastrica occurring in patients with pernicious anemia

### MATERIAL AND METHODS

Ten young, healthy adults who worked in the hospital and who had no evidence of disease, were used as controls in these experiments All of the forty seven patients with pernicious anemia were proven cases, and a more detailed description of them will be found later in the paper

No food or drink was given to the subjects between the evening meal and the morning of the test. Early in the morning the fasting contents were removed by means of a Rehfuess tube, after which 0.5 mgm of histamine hydrochloride was injected All patients developed a generalized erythema following the injection Subsequent samples of the gastric juice were collected at twenty minute intervals over a period of eighty minutes, great care being taken to remove as completely as possible all of the gastric contents at each withdrawal The patients were advised to take particular care not to swallow saliva.

To fourteen of the forty seven patients with pernicious anemia intravenous liver extract was given at the end of the sixty minute period, and two more

samples of gastric juice were collected. Each specimen of gastric juice was measured in a graduated cylinder and was filtered through paper before using it for analysis. The presence or absence of bile, blood, and mucus was noted.

The pH was determined by means of the colorimetric method of Brown (4) using one drop of gastric juice, the free acid by Sahli's method using 1 cc of juice, and the total acid on 1 cc of juice, titrating with N/100 sodium hydroxide using phenolphthalein as an indicator. Chlorides were measured by Wilson and Ball's modification (5) of the Van Slyke method, and nitrogen was determined, using 0.5 cc of gastric juice, by the gasometric method of Van Slyke (6).

Phosphorus was determined by Fiske and Subbarow's method (7). One-half cc of the samples of gastric juice obtained from patients with pernicious anemia were digested in pyrex test tubes graduated to a volume of 10 cc. Larger samples, depending upon the acidity, were used when the subjects were normals. When normal juice was precipitated with trichloroacetic acid, the filtrate contained practically the same amount of phosphorus as the whole juice, whereas, with samples from patients with pernicious anemia, we were unable to get clear filtrates after trichloroacetic acid precipitation, and, therefore, the total phosphorus was determined on the whole juice.

Pepsin was determined by the method of Koch and McMeekin (8)—a simple, rapid, and accurate method, for which only one cc of gastric juice was required. The substrate employed in this method was coagulated egg white, which is particularly useful in the determination of pepsin in gastric juice, since trypsin is not able to act upon it to an appreciable extent.

The coagulated egg white was prepared as follows: a 20 per cent solution of Merck's powdered egg white (or albumin) was prepared and coagulated with heat by placing it in a boiling water bath for fifteen minutes, stirring vigorously throughout the coagulation period. The coagulated egg white was filtered through cheese cloth, and was broken up into small pieces by passing it through a fine meat chopper. It was then shaken with an excess of 0.3 per cent hydrochloric acid for two hours, at 40° C, to remove inorganic salts, colored extractives, and other substances which are soluble in 0.3 per cent hydrochloric acid, thereby reducing the soluble nitrogen blank of the egg white. The coagulated egg white was filtered through gauze with the aid of suction and was thoroughly washed with distilled water. It was then spread out on trays and dried in a current of warm air. After drying, it was ground so as to pass through an eighty mesh sieve.

To determine the amount of peptic activity, 4 grams of egg white were suspended in 99 cc of 0.3 per cent hydrochloric acid in a 125 cc Erlenmeyer flask, at room temperature. Then 1 cc of gastric juice was added. The solution was shaken and placed in an incubator at 40° C for two hours where it was rotated vertically during the entire period of incubation<sup>1</sup>. After the two hour incubation period, the solution was filtered by decantation through 18 cm quantitative filter paper. The refractive index was determined at 25° C on the filtrate by means of an emersion refractometer, and the readings were converted into "milligrams of pepsin" (1 to 4,000) by means of a table prepared by Koch and McMeekin (8). Since the concentration of pepsin is so low in gastric juice obtained from patients with pernicious anemia, only two grams of egg white were used as a substrate, and the total volume of acid was made up to 50 cc instead of 100 cc. Otherwise, the method was identical with that used with normal juice.

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<sup>1</sup> The water bath and shaker, designed by Doctor Koch, was obtained from the Arthur H. Thomas Company, Philadelphia.

Rennin was determined by a method similar to the one described by Michaelis (1). Forty five cc. of fresh, raw cows milk and 5 cc. of a 10 per cent solution of crystalline calcium chloride were mixed. Test tubes containing 2.5 cc. of this reagent were placed in the water bath at 40° C, and, when they had attained the temperature of the bath, gastric juice equivalent to 1/100 cc to 1 cc., depending upon the rennet activity of the juice, was added to the tubes and the clotting time noted. The rennet activity was converted into "milligrams of rennin" by referring to a table made by using the above technique on known concentrations of dried commercial rennin. The clotting time with the known concentrations of rennin are shown in Table 1.

TABLE 1  
*The clotting time required for known amounts of dry commercial rennin*

Rennin mgm.	Average clotting time seconds	Average clotting time minutes
1.5	9	
1.0	10.5	
0.5	21	
0.3	31	
0.2	44	
0.1	73	
0.075	98	
0.050	145	1.63
0.030	222	3.70
0.020	334	5.90
0.010	650	10.83

#### RESULTS IN NORMAL SUBJECTS

In Table 2 are tabulated the results of the analyses of the gastric juice obtained from ten normal subjects.

In Chart I, the values obtained in an average normal subject are plotted. With the exception of one case, the fasting samples contained only a trace of mucus. After histamine stimulation, the juice became more watery, filtering with the greatest rapidity at the height of the acid secretion. The greatest volumes of juice were obtained at the twenty minute period and ranged from 35 to 80 cc., with an average of 56 cc.

In the fasting samples the pH varied from 1.6 to 7.2. The peak of the acid secretion was in the forty minute period in the majority of cases, with pH values of 1.1 or less. The free acid at its peak ranged from 72 to 140 cc. N/10 per 100 cc., and the total acid varied from 77 to 149.

The pepsin and rennin were secreted at the same rate, with the peak at twenty minutes. At the height of secretion the pepsin varied from 1.4 to 7.1 mgm. per cc., with an average of 4.6 mgm., and the rennin varied from 15 to 70 mgm. The concentration of enzymes in the gastric juice did not follow the curve of acid secretion.

The nitrogen concentration fell after stimulation, which is in accord with the data in the literature. The lowest values for nitrogen were found at the peak of the acid secretion. The curve of the nitrogen concentration did not follow that of the enzymes. This is contrary to the findings of Pollard and Bloomfield (9), who stated that in normal persons the con-

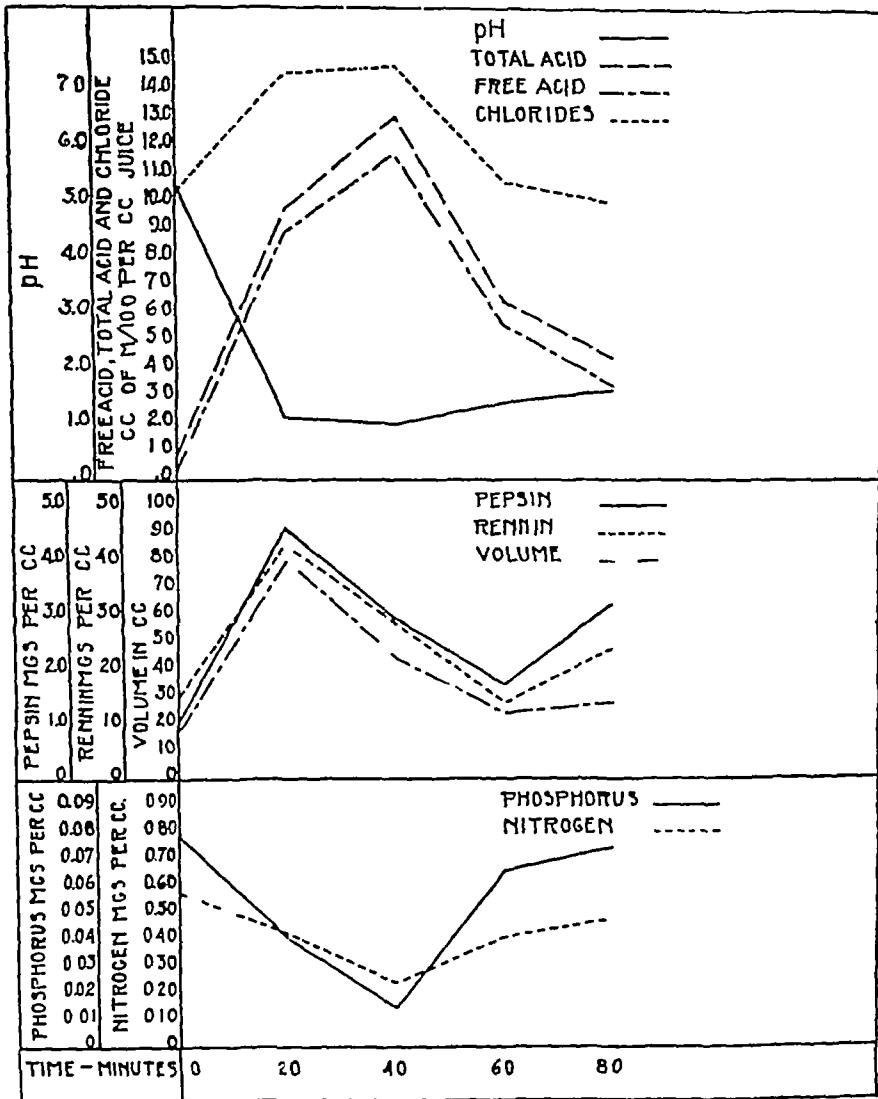
TABLE 2  
The volumes, pH values, free and total acid, pepsin, rennin, nitrogen, chlorides, and phosphorus values of the gastric juice of ten young healthy adults taken at twenty minute intervals following the injection of 0.5 mgm of histamine hydrochloride subcutaneously

Case number	Specimen	Bile	Blood	Mucus	pH	Volume	Total acid per specimen	Free acid per specimen	Free acid per cc	Pepsin	Total pepsin	Rennin	Total rennin	Nitrogen	Total nitrogen	Chlorides	Total chlorides	Phosphorus	Total phosphorus
						cc	cc N/100 per cc	cc N/100 per cc	cc N/100 per cc	mgm per cc	mgm	mgm per cc	mgm	mgm per cc	mgm	mgm cc	mgm	mgm per cc	mgm
1	Fasting	—	—	—	4.0	21	1.4	29	0.8	0.6	13	8	168	0.57	12	2.7	59	0.127	2.7
	20'	—	—	—	1.4	76	6.7	506	5.2	1.4	106	14	1026	0.37	28	3.9	298	0.052	3.9
	40'	—	—	—	1.0	61	14.9	909	10.9	1.8	110	17	1037	0.19	12	5.4	326	0.020	1.3
	60'	—	—	—	1.2	45	11.7	525	8.7	1.3	59	10	450	0.22	10	3.6	164	0.038	1.7
2	80'	—	—	—	1.4	23	6.1	140	5.1	0.9	21	8	188	0.31	7	3.1	70	0.067	1.6
	Fasting	Tr	—	—	3.2	14	2.4	33	1.6	1.8	25	10	140	0.43	6	2.9	40	0.067	0.9
	20'	Tr	—	—	1.2	37	10.9	403	9.4	7.1	279	69	2371	0.43	16	5.2	190	0.021	0.8
	40'	—	—	—	1.0	62	12.4	765	11.2	3.2	196	24	1488	0.23	14	5.3	330	0.018	1.1
3	60'	—	—	—	1.0	27	11.7	315	10.0	2.8	75	21	567	0.24	7	4.8	129	0.018	0.5
	80'	—	—	—	1.0	23	10.5	242	8.9	3.8	88	29	667	0.32	7	4.6	106	0.020	0.5
	Fasting	—	—	++	6.0	8	—	—	0.0	1.7	13	18	144	—	—	—	—	—	—
	20'	—	—	—	1.1	43	9.5	409	8.2	4.3	186	60	2580	0.46	21	5.1	226	0.029	1.3
4	40'	—	—	—	1.0	56	11.7	678	9.8	3.2	181	30	1680	0.32	14	5.2	291	0.018	1.0
	60'	—	—	—	1.2	8	8.5	66	7.5	3.1	24	—	—	0.41	3	4.4	35	0.029	0.2
	80'	—	—	—	2.0	11	3.8	42	2.9	2.5	28	22	242	0.55	6	3.6	40	0.057	0.6
	Fasting	Tr	—	+	5.3	18	0.9	17	0.3	1.1	20	15	270	0.58	10	3.7	67	0.078	1.4
5	20'	—	—	—	1.1	80	9.8	764	8.9	0.6	368	43	3440	0.43	34	5.2	416	0.040	3.2
	40'	—	—	—	1.0	44	13.1	576	11.8	2.9	128	29	1276	0.24	11	5.4	238	0.014	0.7
	60'	—	—	—	1.4	14	6.4	100	5.7	1.8	25	14	196	0.41	6	3.8	53	0.066	0.8
	80'	—	—	—	1.6	18	4.5	80	3.3	3.2	58	24	432	0.48	9	3.6	65	0.074	1.3
5	Fasting	Tr	—	—	1.6	55	4.9	270	4.3	2.9	160	35	1925	0.50	27	3.7	205	0.074	4.1
	20'	—	—	—	1.2	48	8.1	389	7.6	7.2	346	60	2880	0.50	24	4.4	211	0.044	2.1
	40'	—	—	—	1.0	52	11.9	619	11.3	6.2	322	48	2496	0.35	18	5.0	260	0.029	1.5
	60'	—	—	—	1.0	60	10.6	636	10.0	4.8	288	32	1920	0.34	19	4.6	276	0.040	2.4
5	80'	—	—	—	1.0	35	13.5	473	13.3	7.0	245	70	2450	0.31	11	6.6	196	0.006	0.2

TABLE 2 (continued)

Case number	Specimen	Bile	Blood	Mucus	pH	Volume	Total acid per spec. cc.	Free acid per cc.	Free acid per spec. men	Pepsin	Total pepsin	Rennin	Total rennin	Nitrogen	Total nitrogen	Chlorides	Total chlorides	Phosphorus	Total phosphorus
						cc.	cc. N/100 per cc.	cc. N/100 per cc.	cc. N/100	mgm. per cc.	mgm	mgm per cc.	mgm	mgm per cc.	mgm	mgm cc.	mgm	mgm per cc.	mgm
6	Fasting	+	—	—	2.1	52	2.8	147	2.0	104	3.1	161	24	0.62	32	4.1	213	0.052	2.7
	20'	Tr	—	—	1.4	68	5.0	340	4.3	292	4.1	279	34	0.42	29	4.3	292	0.028	1.6
	40'	Tr	—	—	1.1	58	7.7	447	7.2	418	2.9	168	22	0.35	20	4.2	244	0.029	1.7
	60'	+	—	—	1.1	45	8.6	387	8.1	364	3.1	137	27	0.34	15	4.5	203	0.019	0.9
7	80'	Tr	—	—	1.6	17	3.7	63	3.3	56	3.0	51	26	0.41	7	3.5	60	0.035	0.6
	Fasting	Tr	—	+	7.0	48	0.3	14	0.0	0	2.4	115	7	0.61	29	3.0	144	0.136	6.5
	20'	—	—	—	1.2	62	9.5	589	8.2	570	5.4	335	68	0.41	29	5.1	316	0.040	2.4
	40'	—	—	—	1.0	56	14.1	790	14.0	784	3.9	220	42	0.20	11	5.8	326	0.012	0.6
8	60'	—	—	—	1.0	65	13.4	871	13.3	864	3.0	195	40	0.18	11	5.6	366	0.016	1.0
	80'	—	—	—	1.0	34	14.3	486	13.8	469	3.4	116	34	0.23	8	5.6	190	0.011	0.4
	Fasting	—	—	—	7.2	27	0.3	8	0.0	0	0.2	5	4	0.56	15	3.3	88	0.081	2.2
	20'	—	—	—	1.4	47	5.6	263	4.9	230	4.2	197	44	0.55	26	4.4	207	0.080	3.8
9	40'	—	—	—	1.1	46	8.7	420	8.0	368	3.4	156	35	0.44	21	4.5	208	0.021	1.3
	60'	—	—	—	1.3	23	7.0	161	6.3	145	2.9	67	33	0.55	12	4.2	97	0.040	0.9
	80'	—	—	—	1.7	14	3.8	53	3.0	42	2.8	39	22	0.64	9	3.7	52	0.066	0.9
	Fasting	Tr	—	+	7.1	52	0.1	5	0.0	0	0.6	31	2	0.55	28	2.8	146	0.068	3.5
10	20'	—	—	—	1.9	35	3.2	112	2.6	91	1.6	56	15	0.53	18	3.3	116	0.052	1.8
	40'	—	—	—	1.1	46	9.6	442	8.5	391	2.0	92	19	0.40	18	4.5	207	0.047	2.2
	60'	—	—	—	1.0	52	11.5	598	10.4	541	2.3	120	18	0.36	18	4.8	250	0.038	2.0
	80'	Tr	—	—	1.2	31	9.0	279	7.8	242	1.6	50	15	0.32	10	4.0	124	0.059	1.8
	Fasting	—	—	—	2.0	43	4.9	201	3.3	142	6.7	288	71	0.81	35	3.2	138	0.103	4.4
	20'	—	—	—	1.3	70	6.2	434	5.5	375	6.2	434	62	0.44	31	3.5	243	0.052	3.6
	40'	—	—	—	1.1	51	10.1	515	9.5	485	4.4	224	45	0.36	18	4.3	220	0.037	1.9
	60'	—	—	—	1.2	29	8.4	244	7.6	220	3.5	102	30	0.33	10	3.7	108	0.048	1.4
	80'	—	—	—	1.0	100	10.3	1030	10.1	1010	4.3	430	35	0.30	30	4.4	440	0.037	3.7

centration of pepsin falls markedly after stimulation and follows closely the curve of nitrogen concentration. In all but one of our normal individuals, there was an increase in enzyme concentration after histamine stimulation, whereas the nitrogen values were always lowered. The



VALUES FROM GASTRIC JUICE OF NORMAL SUBJECT NUMBER 4 (A TYPICAL NORMAL) FOLLOWING THE SUBCUTANEOUS INJECTION OF 0.5 MG. OF HISTAMINE HYDROCHLORIDE

secretion of enzymes apparently influenced the nitrogen concentration. In Case 2, Case 5, and Case 8, after stimulation, there was the usual increase in acid, with a simultaneous increase in pepsin and rennin of greater proportions than usual, but with a comparatively smaller decrease in nitrogen concentration.

The total chloride concentration, in general, followed the acid concentration, however, the increase in chlorides was not in proportion to the change in acidity, and could not, in itself, account for the increased acidity. Gamble and McIver (10) and Austin and Gammon (11), using dogs with gastric pouches, stated that the chloride concentration remained almost constant during their experiments.

The total phosphorus concentration decreased as the acidity increased, reaching its low point at the height of the acid secretion. As the acid decreased, the phosphorus values returned to values approaching those of the fasting samples. Bulger, Stroud, and Heideman (12) point out that, if much acid is produced, the phosphates decrease, and they attribute the fluctuations to relative amounts of secretion from the mucous glands. We have also found that if stimulation did not produce a secretion of free hydrochloric acid, the concentration of phosphate often increased. Hoesch (13), too, showed that the phosphorus content was low when the acidity was high, and, in cases of anacidity or achylia, the phosphorus content of the gastric juice was high. Hoesch believes that the antagonism between chloride and phosphate may be an important factor in the secretory mechanism that produces free hydrochloric acid.

Bolton and Goodhart (14) believe that mucus is an important factor in controlling the acidity of the stomach, especially when there is only a small flow of gastric juice. Since the nitrogen and phosphorus of the gastric juice are probably secreted mainly by the mucous glands, the fall in both nitrogen and phosphorus with active acid secretion combined with their subsequent rise as the acid secretion diminishes, might further point to an important rôle of gastric mucus in the control of the acidity of the stomach.

#### RESULTS IN PERNICIOUS ANEMIA

Table 3 records the results of the forty seven individual analyses, while Chart II shows the average of the results obtained in the forty-seven cases of pernicious anemia. The gastric juice obtained in patients with pernicious anemia was usually small in volume and contained much mucus, but the samples with the greatest volumes usually contained the least mucus. Vineburg and Babkin (15), and Gilman and Cowgill (16) stated that histamine did not stimulate the gastric enzymes but that pilocarpine did. We did separate, complete gastric analyses on one patient, after 0.5 mgm. histamine given subcutaneously, after 3.2 mgm. pilocarpine subcutaneously, and after 20 cc. of liver extract intravenously. There was practically no difference in the three analyses, so we considered histamine an adequate stimulus. Two patients also had gastric analyses after histamine stimulation, first while in relapse and again soon after remission had been induced by liver extract, and there were no differences in the gastric findings.



TABLE 3

The volumes, pH values, total acid, pepsin, rennin, nitrogen, chlorides, and phosphorus values of the gastric juice of forty-seven patients with pernicious anemia, withdrawn at twenty minute intervals following the injection of 0.5 mgm of histamine hydrochloride subcutaneously

Case number	Specimen	Bile	Blood	Mucus	pH	Volume cc	Total acid per specimen cc	Free acid per specimen cc	Free acid per specimen cc	Pepsin mgm per cc	Total pepsin mgm	Rennin	Nitrogen mgm per cc	Total nitrogen mgm	Chlorides mgm per cc	Total chlorides mgm	Phosphorus mgm per cc	Total phosphorus mgm
1	Fasting																	
	20'	-	+	++	7.5	14	0.5	7	0	0.04	0.6		0.89	12				
	40'	-	+	+	7.5	16	0.5	8	0	0.04	0.6		1.02	16				
	60'	-	+	+	7.5	9	0.6	5	0	0.07	0.6		1.34	12				
2	Fasting																	
	20'	-	-	++	7.1	8	0.5	4	0	0.03	0.2		0.87	7				
	40'	-	+	+	7.1	9	0.5	4	0	0.02	0.2		0.75	7				
	60'	+	+	+	7.2	14	0.8	12	0	0.06	0.9		1.16	17				
3	Fasting																	
	20'	-	-	++	7.8	16	0.2	3	0	0.03	0.5		0.73	12				
	40'	-	-	++	7.8	7	0.2	1	0	0.03	0.2		0.62	4				
	60'	-	-	+	7.8	4	0.3	1	0	0.02	0.1		0.33	1				
4	Fasting																	
	20'	-	-	++	8.2	27	0.2	5	0	0.02	0.2		0.97	25				
	40'	-	-	++	8.8	5	0.2	1	0	0.03	0.2		0.75	4				
	60'	-	+	++	8.0	7	0.2	1	0	0.02	0.2		0.65	4				
5	Fasting																	
	20'	-	-	++	8.0	5	0.2	1	0	0.02	0.1		0.54	2				
	40'	-	-	++	8.6	7	0.2	1	0	0.03	0.2		1.06	8				
	60'	-	-	++	8.0	35	0.4	14	0	0.07	2.1		1.37	48				
	Fasting																	
	20'	-	+	+	7.8	24	0.5	12	0	0.06	1.5		1.19	29				
	40'	-	+	+	7.6	19	0.2	4	0	0.08	1.6		0.07	21				
	60'	-	+	+	8.0	23	0.3	14	0	0.05	1.2		0.81	19				

TABLE 3 (continued)

Case number	Specimen	Bile	Blood	Mucus	pH	Volume cc.	Total acid per spec. cc.	Free acid per spec. cc.	Free acid per spec. men	Pepsin	Total pepsin	Rennin	Nitrogen	Total nitrogen	Chlorides	Total chlorides	Phosphorus	Total phosphorus
							cc N/100 per cc	cc N/100 per cc	cc N/100 per spec. men	mgm per cc	mgm per cc		mgm per cc	mgm per cc	mgm per cc	mgm per cc	mgm per cc	mgm
6	Fasting				7.6	12	0.8	0	0	0.05	0.6		0.10	12				
	20'	+	-	+	7.8	12	0.6	0	0	0.05	0.6		0.97	12				
	40'	+	-	+	7.8	18	0.5	0	0	0.05	1.0		0.73	13				
	60'	+	-	+	7.8	10	0.3	0	0	0.04	0.4		0.71	7				
	80'	+	-	+	7.6	8	0.5	0	0	0.05	0.4		0.87	7				
7	Fasting				7.5	8	0.4	0	0	0.05	0.4		0.45	3				
	20'	+	-	+	7.4	14	0.4	0	0	0.05	0.8		0.32	4				
	40'	+	-	+	7.5	5		0	0	0.04	0.2		0.34	2				
	60'	+	-	+	7.4	5		0	0	0.04	0.2		0.42	2				
8	Fasting		+	+	7.8	3		0	0	0.04	0.1				1.8	12		
	20'	-	+	+	7.8	7		0	0	0.12	0.9							
	40'	-	+	+	7.8	2		0	0	0.15	0.3							
	60'	-	+	+	7.9	5		0	0	0.08	0.4							
	80'	-	+	+		2		0	0	0.15	0.3							
9	Fasting		-	-	8.1	5		0	0	0.00	0.0		0.62	4				
	20'	+	-	-	8.0	5		0	0	0.00	0.0							
	40'	+	-	-	7.9	7		0	0	0.00	0.0							
	60'	-	-	-	7.9	3		0	0	0.00	0.0		0.58	3		10		
	80'	-	-	-	7.5	5		0	0	0.00	0.0							
10	Fasting				7.5	13	0.7	0	0	0.01	0.1		0.78	10		35		3.6
	20'	+	-	-	7.7	34	0.3	0	0	0.00	0.0		0.82	28		50	0.11	
	40'	Tr	-	+	7.7	26	0.5	0	0	0.00	0.0		0.60	15		38	0.10	
	60'	-	+	+	7.7	18	0.3	0	0	0.00	0.0		0.40	7		21	0.12	
	80'	-	+	+	7.6	2		0	0	0.00	0.0							2.1

TABLE 3 (continued)

Case number	Specimen	Bile	Blood	Mucus	pH	Volume	Total acid per cc.	Free acid per cc.	Free acid per specimen	Pepsin	Total pepsin	Rennin	Nitrogen	Total nitrogen	Chlorides	Total chlorides	Phosphorus	Total phosphorus
						cc	$\frac{cc}{N/100}$ per cc	$\frac{cc}{N/100}$ per cc	$\frac{cc}{N/100}$	$\frac{mgm}{cc}$	$\frac{mgm}{cc}$		$\frac{mgm}{cc}$	$\frac{mgm}{cc}$	$\frac{mgm}{cc}$	$\frac{mgm}{cc}$	$\frac{mgm}{cc}$	$\frac{mgm}{cc}$
11	Fasting	—	—	—	8.1	8	2	0	0	0.02	0.1	Tr	0.66	5	2.0	16		
	20'	—	—	+	7.8	40	16	0	0	0.02	0.8		0.89	36	2.3	94		
	40'	—	—	—	7.4	14	7	0	0	0.02	0.2		0.91	13	2.1	29		
	60'	—	—	—	7.6	13	5	0	0	0.03	0.4		0.92	9				
	80'	—	+	—	7.5	10	3	0	0	0.03	0.3							
12	Fasting	—	—	++	8.0	10	2	0	0	0.00	0.0	0	0.33	3	2.1	21		
	20'	—	—	++	7.7	15	5	0	0	0.02	0.3	0	0.95	14	1.8	27		
	40'	—	—	++	7.6	10	4	0	0	0.03	0.3	0	0.94	9	1.8	18		
	60'	—	+	++	7.7	10	5	0	0	0.02	0.2	0	0.75	8	2.7	27		
	80'	—	—	++	8.0	2		0	0	0.01	0.1	0						
13	Fasting	+	+	—	8.0	29	6	0	0	0.03	0.9	0	1.12	32	3.1	89		
	20'	Tr	+	—	8.4	42	8	0	0	0.03	1.3	0	2.10	88	2.2	96		
	40'	Tr	+	—	8.2	21	4	0	0	0.03	0.6	0	2.34	49	1.9	39		
	*60'	—	+	+	7.4	11	6	0	0	0.05	0.6	0	1.02	11	2.1	23		
	80'	++	—	—	7.5	29	38	0	0	0.05	1.5	0	1.62	47	2.2	63		
14	100'	Tr	—	—	7.3	16	14	0	0	0.03	0.4		1.08	17	2.3	37		
	Fasting	—	—	—	8.2	19	0	0	0	0.02	0.4		0.68	13	2.4	45	0.11	2.0
	20'	—	—	—	8.2	42	1	0	0	0.00	0.0	Tr	0.82	34	2.0	84	0.14	5.7
	40'	—	—	—	8.1	19	7	0	0	0.00	0.0		0.38	9	1.7	32	0.17	3.3
	*60'	—	—	—	8.1	4	1	0	0	0.04	0.2							
	80'	—	—	—	8.1	45	9	0	0	0.05	2.3	Tr	1.40	63	2.3	104	0.14	6.1
	100'	—	—	—	7.4	20	8	0	0	0.07	1.4		1.14	23	2.4	45	0.19	3.7

TABLE 3 (continued)

Case number	Specimen	Bile	Blood	Mucus	pH	Volume	Total acid per cc.	Total acid per spec. men.	Free acid per cc.	Free acid per spec. men.	Pepsin	Total pepsin	Remnin	Nitrogen	Total nitrogen	Chlorides	Total chlorides	Phosphorus	Total phosphorus
						cc.	cc. N/100 per cc.	cc. N/100 per spec. men.	cc. N/100 per cc.	cc. N/100 per spec. men.	mgm. per cc.	mgm. per spec. men.		mgm. per cc.	mgm. per spec. men.	mgm. per cc.	mgm. per spec. men.	mgm. per cc.	mgm. per spec. men.
15	Fasting	—	—	Tr	8.0	45	0.2	9	0	0	0.00	0.0	0	0.53	23	1.4	65	0.17	7.5
	20'	—	—	—	8.1	35	0.2	7	0	0	0.01	0.3	0	0.64	22	1.0	35	0.19	6.9
	40'	—	—	—	7.2	14	0.5	7	0	0	0.01	0.1	0	0.70	9	1.2	16	0.22	2.9
	*60'	—	—	—	7.3	28	0.4	12	0	0	0.01	0.2	0	0.52	11	1.2	33	0.21	5.8
	80'	—	—	—	7.3	15	0.4	6	0	0	0.03	1.4	0	0.65	10	1.4	21	0.20	3.0
16	100'	—	—	—	7.4	20	0.4	8	0	0	0.03	0.6	0	0.53	11	1.5	30	0.19	3.7
	Fasting	Tr	Tr	—	8.2	14	0.2	3	0	0	0.00	0.0	0	0.92	13	2.7	38	0.13	1.8
	20'	—	Tr	++	8.2	17	0.3	5	0	0	0.03	0.4	0	1.17	19	2.9	49	0.10	1.7
	40'	—	—	++	7.7	16	0.6	9	0	0	0.01	0.2	0	0.77	12	2.0	32	0.16	2.6
	*60'	—	—	+	7.7	3			0	0	0.02	0.1		0.60	7	1.7	20	0.15	1.8
17	80'	—	++	—	7.5	12	0.5	6	0	0	0.02	0.2		0.65	8				
	100'	—	—	—	7.6	5			0	0	0.03	0.1							
	Fasting	—	—	++	8.2	11	0.0	0	0	0	0.02	0.1		0.81	9	2.8	30	0.12	1.3
	20'	—	—	++	8.2	37	0.1	4	0	0	0.03	0.4	0	1.10	41	2.8	105	0.11	4.2
	40'	++	—	—	8.0	16	0.4	6	0	0	0.02	0.4	Tr	1.76	27	3.0	46	0.15	2.3
18	60'	—	+	—	7.5	5	0.6	3	0	0	0.01	0.1		1.15	10				
	80'	Tr	—	—	7.3	9	0.6	5	0	0	0.00	0.0	Tr						
	Fasting	++	—	+	8.2	37	0.0	0	0	0	0.04	1.4	Tr	2.95	106	2.7	100	0.15	5.4
	20'	+	—	+	8.4	83	0.0	0	0	0	0.01	0.8	Tr	1.24	103	1.8	150	0.11	9.1
	40'	—	—	++	8.6	62	0.0	0	0	0	0.00	0.0	0	0.74	46	1.7	107	0.10	6.2
	60'	—	—	++	8.4	48	0.0	0	0	0	0.00	0.0	Tr	0.44	21	1.4	66	0.13	6.2
	80'	—	—	++	8.4	26	0.0	0	0	0	0.00	0.0	0	0.69	18	1.0	26	0.14	3.5

TABLE 3 (continued)

Case number	Specimen	Bile	Blood	Mucus	pH	Volume cc	Total acid per cc N/100	Total acid per specimen cc N/100	Free acid per cc N/100	Free acid per specimen cc N/100	Pepsin mgm per cc	Total pepsin mgm	Rennin	Nitrogen mgm per cc	Total nitrogen mgm	Chlorides mgm per cc	Total chlorides mgm cl cc	Phosphorus mgm per cc	Total phosphorus mgm
19	Fasting	++	—	—	7.2	9			0	0	0.03	0.3	Tr						
	20'	++	—	—	7.3	4			0	0	0.03	0.1	Tr						
	40'	++	—	—	7.3	4			0	0	0.13	0.5	Tr						
	80'	++	—	—	7.0	5			0	0	0.17	0.9							
20	Fasting	++	—	+	8.1	14			0	0	0.06	0.8							
	20'	++	—	+	8.0	13			0	0	0.05	0.7							
	40'	Tr	+	++	8.2	8			0	0	0.07	0.6							
	60'	Tr	+	++	8.2	11			0	0	0.07	0.8							
21	Fasting	—	—	+	8.0	9			0	0	0.02	0.2	.						
	20'	—	—	+	7.8	7		4	0	0	0.06	0.5	0						
	40'	—	—	+	7.2	5			0	0	0.06	0.3	0						
	*60'	+	—	—	7.2	3			0	0	0.06	0.2							
	80'	Tr	—	—	7.1	9		5	0	0	0.06	0.5	Tr						
22	100'	—	+	—	7.2	2			0	0	0.14	0.3							
	Fasting	—	—	—	7.6	5			0	0	0.07	0.3							
	20'	+	+	—	7.7	4			0	0	0.06	0.2							
	40'	—	+	—	7.7	3			0	0	0.12	0.4							
	*60'	—	—	—	8.0	1			0	0	0.08	0.1							
23	80'	+	—	—	7.7	8			0	0	0.06	0.5							
	Fasting	—	—	—	8.0	17			0	0	0.00	0.0	Tr			1.2	20	0.11	1.8
	20'	—	—	—	8.1	6			0	0	0.05	0.3	Tr					0.10	0.6
	40'	—	—	—	8.2	2			0	0	0.06	0.1	Tr					0.11	0.3
	*60'	—	—	+	8.2	2			0	0	0.04	0.1						0.11	0.3
	80'	+	—	—	8.2	28			0	0	0.00	0.0	Tr			1.5	42	0.08	0.1
	100'	+	—	—	8.3	3			0	0	0.05	0.2	Tr				0.10	0.10	3.0

TABLE 3 (continued)

Case num-ber	Speci-men	Bile	Blood	Mucus	pH	Vol-ume	Total acid per speci-men	Free acid per cc.	Free acid per speci-men	Pepsin	Total pepsin	Rennin	Nitro-gen	Total nitro-gen	Chlo-rides	Total chlo-rides	Phos-phorus	Total phos-phorus
						cc.	cc. N/100	cc. N/100	cc. N/100	mgm. per cc.	mgm.		mgm. per cc.	mgm.	mgm. per cc.	mgm. cl. cc.	mgm. per cc.	mgm.
24	Fasting	-	-	+	8.2	11	0	0	0	0.00	0.0	0						
	20'	-	-	+	7.8	3	0	0	0	0.07	0.2	0						
	40'	-	-	++	8.0	4	0	0	0	0.08	0.3	0						
	60'	-	-	++	8.2	4	0	0	0	0.08	0.3	0						
25	Fasting	-	-	+	7.4	3	0	0	0	0.01	0.1	0						
	20'	-	-	++	7.4	12	0	0	0	0.01	0.2	0	1.07	13	2.5	28	0.12	15
	40'	-	-	++	7.2	9	0	0	0	0.10	0.9		1.35	12	2.4	20	0.12	10
	60'	-	-	++	7.4	2	0	0	0	0.10	0.2							
26	Fasting	Tr	-	-	8.2	7	0	0	0	0.01	0.1	Tr						
	20'	-	+	-	7.6	9	0	0	0	0.07	0.6	0						
	40'	-	+	-	7.6	5	0	0	0	0.10	0.5							
	60'	-	+	-	8.2	7	0	0	0	0.11	0.8	0						
27	80'	-	+	-	7.6	3	0	0	0	0.15	0.5							
	Fasting	-	-	-	8.0	40	0	0	0	0.09	3.6	Tr	0.76	31	3.6	143	0.08	32
	20'	-	-	-	8.2	35	0	0	0	0.06	2.1	0	0.67	23	2.7	95	0.08	2.8
	40'	-	-	-	8.2	19	0	0	0	0.13	2.5	0	0.87	17	2.9	54	0.08	1.5
28	60'	-	-	-	8.1	18	0	0	0	0.08	1.4	Tr	0.84	15	3.1	56	0.10	1.8
	80'	-	-	-	8.2	8	0	0	0	0.00	0.0				2.8	22	0.10	0.8
	Fasting	-	-	+	8.2	6	0	0	0	0.00	0.0	0						
	20'	-	-	+	8.2	15	0	0	0	0.01	0.2	0						
29	40'	-	-	+	7.8	4	0	0	0	0.02	0.1	0						
	60'	+	-	-	8.0	3	0	0	0	0.07	0.2	0						
	80'	++	-	-	8.0	4	0	0	0	0.07	0.3	0						
	80'	++	-	-	8.0	4	0	0	0	0.07	0.3	0						

TABLE 3 (continued)

Case number	Specimen	Bile	Blood	Mucus	pH	Volume	Total acid per specimen	Free acid per specimen	Pepsin	Total pepsin	Rennin	Nitrogen	Total nitrogen	Chlorides	Total chlorides	Phosphorus	Total phosphorus
						cc	cc N/100 per cc	cc N/100 per cc	mgm per cc	mgm		mgm per cc	mgm	mgm per cc	mgm cl cc	mgm per cc	mgm
29	Fasting	-	-	-	7.6	21	0.2	0	0.00	0.0	0	0.44	9	1.4	29	0.13	2.6
	20'	-	-	+	8.5	21	0.0	0	0.05	1.1	0	0.65	14	1.9	40	0.09	1.9
	40'	-	-	-	8.2	4	0.0	0	0.00	0.0	0					0.11	0.4
	*60'	-	+	-	8.0	2	0.0	0	0.04	0.1	0					0.10	0.2
	80'	Tr	-	-	8.2	14	0.0	0	0.04	0.6	0	0.73	10	1.9	27	0.09	1.3
30	100'	+	-	-	7.8	12		0	0.06	0.7	Tr			1.8	22	0.13	1.6
	Fasting	+	-	-	8.4	36	0.0	0	0.07	2.6	Tr	0.78	28	2.5	90	0.12	4.3
	20'	-	-	+	8.3	80	0.0	0	0.07	5.6	0	0.85	68	2.2	178	0.11	8.9
	40'	-	-	+	8.2	28	0.0	0	0.02	0.6	0	0.53	15	1.6	44	0.15	4.2
	*60'	-	Tr	+	8.2	19	0.0	0	0.06	1.1	0	0.53	10	1.5	29	0.16	3.0
31	80'	-	Tr	+	7.8	31	0.0	0	0.08	2.5	Tr	1.02	32	2.0	62	0.16	4.9
	100'	-	Tr	+	8.0	28	0.0	0	0.07	1.8	0	0.56	16	1.9	54	0.13	3.6
	Fasting	+	-	+	8.3	15	0.0	0	0.00	0.0	0	0.69	10	2.0	28	0.11	1.5
	20'	-	-	+	8.3	48	0.0	0	0.09	4.3	0	0.95	46	2.9	142	0.12	5.7
	40'	+	-	-	7.6	26	0.6	16	0.08	2.0	Tr			2.4	63	0.20	5.2
32	60'	+	-	-	7.3	3		0	0.08	0.3				2.2	7	0.17	0.6
	80'	+	-	+	7.6	13	0.1	1	0.09	1.1				2.1	27	0.19	2.4
	Fasting	-	-	+	7.6	4		0	0.03	0.1						0.04	0.2
	20'	-	-	+	8.1	8		0	0.01	0.1	0					0.08	0.6
	40'	-	-	+	8.0	3		0	0.05	0.2							
	60'	-	-	+	8.0	2		0	0.06	0.1	Tr						

TABLE 3 (continued)

Case num ber	Speci- men	Bile	Blood	Mucus	pH	Vol ume cc.	Total acid per cc. $\frac{cc. N/100}{per cc.}$	Total acid per spec. men $\frac{cc. N/100}{per spec. men}$	Free acid per cc. $\frac{cc. N/100}{per cc.}$	Free acid per spec. men $\frac{cc. N/100}{per spec. men}$	Pepsin $\frac{mgm. per cc.}{mgm. per cc.}$	Total pepsin $\frac{mgm. per cc.}{mgm. per cc.}$	Rennin	Nitro- gen $\frac{mgm. per cc.}{mgm. per cc.}$	Total nitro- gen $\frac{mgm. per cc.}{mgm. per cc.}$	Chlo- rides $\frac{mgm. per cc.}{mgm. per cc.}$	Total chlo- rides $\frac{mgm. per cc.}{mgm. per cc.}$	Phos- phorus $\frac{mgm. per cc.}{mgm. per cc.}$	Total Phos- phorus $\frac{mgm. per cc.}{mgm. per cc.}$
33	Fasting	+	-	-	8.2	15	0.2	3	0	0	0.00	0.0	Tr			2.2	34	0.12	18
	20'	+	-	-	8.2	35	0.2	7	0	0	0.05	1.8	Tr			1.6	57	0.12	40
	40'	-	-	-	7.8	23	0.3	7	0	0	0.00	0.0	0			1.4	32	0.13	28
	*60'	++	-	-	7.4	30	0.6	18	0	0	0.00	0.0				1.9	56	0.15	45
	80'	++	-	-	7.6	46	0.5	23	0	0	0.01	0.5				2.1	97		
34	100'	Tr	-	-	7.7	24	0.3	7	0	0	0.01	0.2	Tr			1.1	27	0.14	3.3
	Fasting	-	-	++	8.2	13			0	0	0.01	0.1	0			2.2	29	0.08	1.0
	20'	-	-	++	8.3	35			0	0	0.03	0.9	0			2.4	83	0.05	2.0
	40'	-	-	++	8.1	16			0	0	0.00	0.0	0			2.4	38	0.03	1.3
	*60'	-	-	++	8.1	10			0	0	0.02	0.2	0			2.2	22	0.08	0.8
35	80'	-	-	+	7.5	21			0	0	0.03	0.6	0			2.4	50	0.08	1.8
	100'	-	-	++	8.3	19			0	0	0.00	0.0	0			2.1	41	0.10	1.9
	Fasting	-	-	-	8.0	50	0.2	10	0	0	0.01	0.5	Tr	0.59	30	1.2	58	0.10	5.0
	20'	-	-	-	8.0	30	0.2	6	0	0	0.04	1.2	Tr	0.82	25	1.6	49	0.12	3.6
	40'	-	-	-	7.6	13	0.3	4	0	0	0.05	0.6	Tr	0.73	10	1.3	17	0.18	2.2
36	60'	-	-	-	7.4	11	0.4	4	0	0	0.05	0.5	Tr	0.63	7	1.3	15	0.20	2.2
	80'	-	-	-	7.3	9	0.3	3	0	0	0.05	0.5	0	0.60	5	1.3	12	0.20	1.8
	Fasting	-	-	+	8.4	4	0.0	0	0	0	0.01	0.1						0.01	0.4
	20'	-	-	-	8.5	36	0.0	0	0	0	0.01	0.4	0					0.12	
	40'	-	-	+	8.6	6	0.0	0	0	0	0.03	0.2							4.6
	60'	-	+	+	8.6	5	0.0	0	0	0	0.00	0.0							



TABLE 3 (continued)

Case number	Specimen	Bile	Blood	Mucus	pH	Volume	Total acid per cc.	Total acid per specimen	Free acid per cc.	Free acid per specimen	Pepsin	Total pepsin	Rennin	Nitrogen	Total nitrogen	Chlorides	Total chlorides	Phosphorus	Total phosphorus
						cc	cc N/100 per cc	cc N/100	cc N/100 per cc	cc N/100	mgm per cc	mgm		mgm per cc	mgm	mgm per cc	mgm cl cc	mgm per cc	mgm
37	Fasting	—	—	—	8.2	9	0	0	0	0	0.01	0.1	0	0.82	7	2.0	18	0.11	1.0
	20'	—	Tr	+	8.2	16	0	0	0	0	0.00	0.0	0	0.71	11	1.6	24	0.09	1.5
	40'	—	Tr	—	8.1	14	0	0	0	0	0.04	0.5	0	0.59	8	1.4	20	0.10	1.4
	*60'	—	Tr	—	8.2	2	0	0	0	0	0.00	0.0	0	0.71	16	1.5	34	0.13	3.1
	80'	—	Tr	—	8.0	23	0	0	0	0	0.03	0.7	0	0.51	12	1.4	32	0.12	2.8
38	100'	—	Tr	—	7.4	23	0	0	0	0	0.05	0.4	0	0.51	12	1.4	32	0.12	2.8
	Fasting	—	—	—	8.4	3	0	0	0	0	0.08	1.2	—	—	—	—	—	—	—
	20'	—	—	—	8.4	15	0	0	0	0	0.00	0.0	—	—	—	—	—	—	—
	40'	Tr	—	—	8.4	5	0	0	0	0	0.00	0.0	—	—	—	—	—	—	—
	60'	Tr	—	—	8.0	9	0	0	0	0	0.03	0.3	Tr	—	—	—	—	—	—
39	80'	Tr	—	—	8.0	10	0	0	0	0	0.00	0.0	Tr	—	—	2.8	28	0.09	0.9
	Fasting	—	—	—	7.5	5	0	0	0	0	0.00	0.0	—	—	—	—	—	—	—
	20'	—	—	+	8.0	11	0	0	0	0	0.01	0.1	0	—	—	—	—	0.09	1.0
	40'	—	—	+	7.8	8	0	0	0	0	0.00	0.0	—	—	—	—	—	0.07	0.5
	60'	—	—	+	7.6	2	0	0	0	0	0.00	0.0	—	—	—	—	—	—	—
40	Fasting	—	—	—	7.3	2	0	0	0	0	0.00	0.0	0	—	—	—	—	—	—
	20'	—	—	+	7.6	8	0	0	0	0	0.00	0.0	0	—	—	—	—	—	—
	40'	Tr	—	+	7.5	10	0	0	0	0	0.05	0.5	0	—	—	—	—	—	—
	*60'	Tr	—	+	7.4	2	0	0	0	0	0.05	0.1	0	—	—	—	—	—	—
	80'	+	+	+	8.2	12	0	0	0	0	0.09	1.0	0	—	—	—	—	—	—
	100'	Tr	—	+	7.6	6	0	0	0	0	0.07	0.4	0	—	—	—	—	—	—

TABLE 3 (continued)

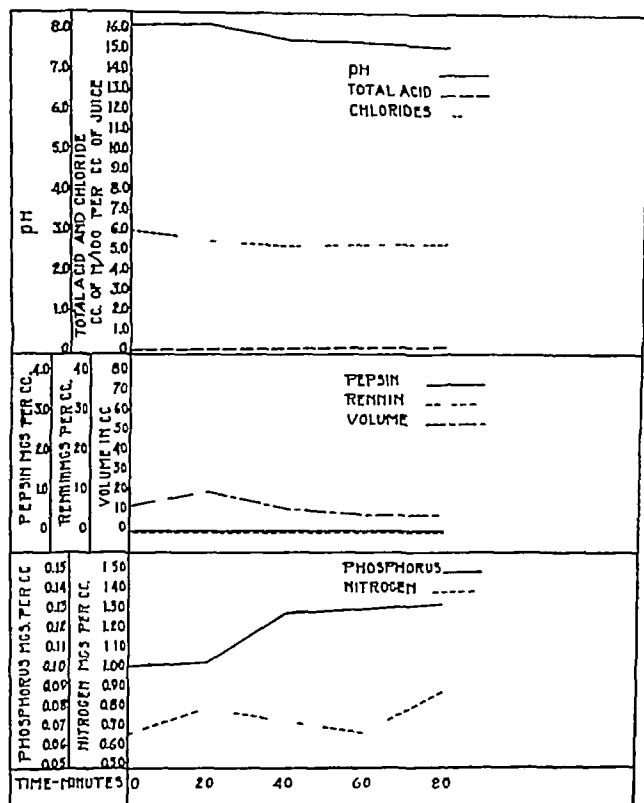
Case num ber	Speci- men	Bile	Blood	Mucus	pH	Vol- ume cc.	Total acid per speci- men cc. N/100 per cc.	Free acid per speci- men cc. N/100 per cc.	Pepsin mgm per cc.	Total pepsin mgm	Rennin	Nitro- gen mgm. per cc.	Total nitro- gen mgm	Chlo- rides mgm. per cc.	Total chlo- rides mgm per cc.	Phos- phorus mgm per cc.	Total phos- phorus mgm
41	Fasting	—	—	—	8.0	9	0	0	0.00	0.0	0	0.72	7	1.8	17	0.12	1.2
	20'	—	—	—	8.4	10	0	0	0.05	0.5	0	0.67	7	1.5	15	0.10	1.0
	40'	—	—	—	8.5	5	0	0	0.06	0.3	0	0.83	4			0.12	0.5
	*60'	—	+	—	8.2	3	0	0	0.05	0.2	0	1.02	3			0.11	0.3
	80'	—	—	—	8.2	13	0	0	0.07	0.9	0	0.88	11	1.3	17	0.12	1.5
42	100'	—	—	—	8.4	7	0	0	0.05	0.4		0.87	6	1.8	13	0.13	0.9
	Fasting	—	—	+	8.2	17	0	0	0.00	0.0	0	0.40	7	2.2	38	0.03	0.5
	20'	—	—	++	7.4	25	0	0	0.06	1.5	Tr	0.52	13	1.9	46	0.08	2.0
	40'	—	—	+	7.2	13	0	0	0.00	0.0	Tr	0.47	6	2.0	26	0.11	1.5
	60'	—	—	+	7.5	5	0	0	0.00	0.0	0					0.12	0.6
43	80'	—	—	+	8.0	9	0	0	0.00	0.0	Tr	0.38	4	1.6	14	0.12	1.0
	Fasting	—	+	++	8.2	3	0	0	0.03	0.1							
	20'	—	+	++	8.3	6	0	0	0.09	0.5							
	40'	—	+	++	8.0	2	0	0	0.15	0.8							
44	60'	—	—	++	8.3	5	0	0									
	Fasting	—	—	+	8.2	17	0	0	0.06	1.0	0	0.43	7	1.8	30	0.08	1.3
	20'	—	—	+	8.0	19	0	0	0.06	1.2	Tr	0.56	10	1.7	31	0.09	1.7
	40'	—	—	+	7.2	18	0.4	0	0.11	1.9	Tr	0.69	12	2.2	39	0.10	1.8
	60'	—	—	+	6.9	27	0.6	0	0.16	4.3	Tr	0.73	20	2.9	78	0.10	2.7
45	80'	—	—	+	8.2	15	0.0	0	0.16	2.4	Tr	0.71	11	2.4	36	0.10	1.5

TABLE 3 (continued)

Case number	Specimen	Bile	Blood	Mucus	pH	Volume	Total acid per specimen cc	Free acid per specimen cc	Free acid per specimen cc	Pepsin	Total pepsin	Rennin	Nitrogen	Chlorides	Total chlorides	Phosphorus	Total phosphorus
						cc	cc N/100 per cc	cc N/100 per cc	cc N/100 per cc	mgm per cc	mgm		mgm per cc	mgm per cc	mgm cl cc	mgm per cc	mgm
45	Fasting	—	—	+	8.6	4	0	0	0	0.00	0.0					0.09	0.4
	20'	Tr	—	+	8.6	10	0	0	0	0.04	0.4	0	0.68	1.0	10	0.11	1.1
	40'	—	—	+	8.3	8	0	0	0	0.11	0.9	0	0.74	1.4	11	0.14	1.1
	60'	—	—	+	8.2	5	0	0	0	0.13	0.7						
46	Fasting	—	—	+	8.4	1	0	0	0	0.00	0.0						
	20'	—	—	+	8.3	14	0	0	0	0.01	0.1	0					
	40'	—	+	+	8.0	4	0	0	0	0.20	0.8	0					
	60'	—	+	+	8.3	6	0	0	0	0.10	0.6						
47	Fasting	+	—	+	7.7	9	0	0	0	0.16	1.4						
	20'	+	—	+	7.8	3	0	0	0	0.09	0.3						
	40'	+	—	+	7.6	13	0	0	0	0.07	0.9						
	60'	+	—	+	7.3	7	0	0	0	0.15	1.1						

\* Patient received the amount of liver extract derived from 100 grams of whole liver, intravenously, immediately following the collection of the sixty minute sample

*Volume* Histamine stimulation caused an increase in volume over the fasting sample in twenty-five of the forty seven cases examined. At the height of secretion, the volume varied from 4 to 83 cc., with an average of 22 cc. The total volume secreted in sixty minutes varied



AVERAGE VALUES FROM GASTRIC JUICE OF FORTY SEVEN PATIENTS WITH PERNICIOUS ANEMIA, FOLLOWING THE SUBCUTANEOUS INJECTION OF 0.5 MGm OF HISTAMINE HYDROCHLORIDE

from 9 to 193 cc., with an average of 41 cc. Following the injection of liver extract there was an increase in volume in all but one of fourteen cases.

The patients who had lower red blood cell counts tended to have smaller volumes than those having higher counts. Seven of eight patients

with red blood cell counts below three million had sixty minute volumes of less than 25 cc. Patients with early or no central nervous system involvement more often had a greater volume of gastric juice than those with moderate or advanced central nervous system involvement. It would seem that the patients with larger volumes more frequently had pH values of the gastric juice that decreased after histamine stimulation, although the volume had no relation to the exact pH value.

The percentage of pepsin in the individual samples bore little or no relationship to the volume, however, the patients with larger total volumes tended to have the higher total pepsin values, although a few of the patients with the highest volumes had the smallest amounts of pepsin. The amounts of rennin present seemed to have no relation to the volume of the sample. Contrary to expectations, the percentage of phosphorus was higher in the samples with the greater volumes. Likewise, in the cases with greater volumes, there was a higher percentage of cases in which there was an increase in phosphorus values over the fasting. The samples having larger volumes usually had a higher percentage of chlorides. The amount of nitrogen in a sample bore no relationship to the volume of the sample.

*Acidity* In all samples there was an absence of free hydrochloric acid and the pH varied from 6.9 to 8.6. Only five of the forty-seven patients had not received liver extract medication previous to the gastric analysis. The length of time the others had been on liver extract medication varied from a few months to five years. This continued absence of free acid in the gastric juice of patients with pernicious anemia, after oral liver therapy, is very well known, and it is interesting to note that the seventeen patients who have received liver extract by injection for from one to eight months still have an achylia gastrica. The amount of total acid excreted in sixty minutes varied between 0 and 30 cc. N/100. In only fourteen cases was there a measurable drop in pH value after histamine stimulation. After liver extract injection there was no constant change in pH value. The presence of bile in the samples caused an increase in the amount of total acid present and a lowering of the pH. There was no relation between the amount of total acid present or the height of the pH and the clinical condition of the patient, except that only one of eight patients with red blood cell counts below 3.0 million had a pH value below 7.5 in any sample. However, in the fourteen instances in which the pH of the gastric juice decreased after histamine, nine of the patients had only early or no central nervous system involvement. The height of the pH bore no relation to the percentage of pepsin, rennin, phosphorus, chlorides, or nitrogen in the samples, but, in the cases with a decrease in pH value after histamine stimulation, there was a greater percentage with an increase in phosphorus, chlorides, and nitrogen.

*Pepsin* The pepsin in the gastric juice was either totally absent or

present in essentially negligible amounts, varying between 0.0 and 0.20 mgm per cc., with an average of 0.04 mgm. The total amount of pepsin secreted in sixty minutes varied between 0.0 and 7.4 mgm., with an average of 1.8. There was a slight increase in the amount of pepsin after histamine stimulation in thirty-three of the forty-seven cases examined. The injection of liver extract caused an increase in pepsin in seven of fourteen cases. The clinical condition of the patient did not seem to show any relationship to the percentage of pepsin present in the samples. Likewise, the amount of pepsin showed no relation to the height of pH, amount of phosphorus, chlorides, or nitrogen, although thirteen of seventeen cases with an increase in nitrogen after histamine also had an increase in pepsin. A trace of bile in the specimen occasionally caused a slight increase in peptic activity, but even then the amount of pepsin found was not over 0.17 mgm per cc. Occasionally, when bile was present, there was a total absence of peptic activity.

*Rennin* The amount of rennin present varied from 0.0 to 0.19 mgm per cc., with an average of 0.013. The total amount of rennin secreted in sixty minutes varied from 0 to 4.0 mgm (average 0.42 mgm). Because of the very small amounts of rennin present—the maximum in the patients with pernicious anemia being only 1/350th of the maximum in the normals—it was decided to express the rennin in Chart II as either being present or absent. The presence of bile in the sample usually caused an increase in amount of rennin, but again there occasionally was no rennin in samples containing bile. There seemed to be no relation between the amount of rennin in the gastric sample and the clinical condition of the patient or the pH value, volume, amount of phosphorus, nitrogen, or chlorides. The determination of rennin was a good check on the pepsin determination, as there was always a proportionately greater decrease in the amount of rennin when there was a decrease in pepsin.

*Nitrogen* The amount of nitrogen present was definitely high in all samples. The presence of bile in the samples caused a great increase in the amount of nitrogen, and, therefore, the nitrogen determinations were of no value in samples containing bile. The amount of nitrogen present in the samples of gastric juice had no relation to the clinical condition of the patient. An increase in the concentration of nitrogen was noted in six of eight patients who received liver extract intravenously. The amount of nitrogen present showed no relationship to pH values or to the amounts of phosphorus, chlorides, pepsin, or rennin.

*Chloride* The chloride values were definitely low, but they did not seem to be as low as would be expected with a total absence of free hydrochloric acid—a few high values, obtained in patients with achylia, were greater than some of the lower values obtained in normal persons. There was an increase in chlorides after histamine stimulation in only nine of twenty-five cases. As has been shown above, there was a tendency

for specimens with larger volumes to have higher percentages of chlorides. There was no correlation between the amounts of chlorides and the pH, amounts of nitrogen, pepsin, rennin, and phosphorus in the samples.

*Phosphorus* The amount of phosphorus present was greatly increased in nearly all specimens. In eighteen of twenty-five cases the phosphorus value increased after histamine stimulation. As has been shown, the specimens with larger volumes more often had higher phosphorus values, and a higher percentage of the specimens with larger volumes had an increase in phosphorus values after histamine stimulation. There was no correlation between the phosphorus values and the pH, amounts of nitrogen, chlorides, pepsin, or rennin.

It is evident from these findings that, while in normal persons there is a direct relationship between the free acid, total acid, chlorides, phosphorus, and nitrogen in the gastric juice after histamine stimulation, there is absolutely no relationship between these substances in the gastric juice of patients with pernicious anemia.

*Gastric juice findings in relation to maintenance dosage of liver extract*

All of the forty-seven patients in this group were clinically and hematologically typical of pernicious anemia, and either had had or subsequently have had good responses to liver extract administered by mouth or by injection. The length of time that these patients have been on liver extract has varied from 0 to 5 years. Twenty-one of the patients have been followed at monthly intervals by this department for from over one to five years, and the remaining patients for only a few months. Seventeen were in relapse (either mild or severe) at the time the analyses were made. The red blood cell counts varied from 0.5 million to 6.0 million and the hemoglobin percentages from 13 to 115 per cent. All degrees of central nervous system involvement were represented—from the very slightest to the most advanced stage where the patient was completely bedridden. In this group of patients, some were able to maintain their blood at normal levels on a very small daily amount of potent liver extract by mouth, while others were not able to do this on large amounts and required liver extract by injection.

An attempt was made to determine whether the quantity or quality of the gastric juice bore any relation to the amount of liver extract required to maintain the blood at normal levels. There were thirty-one patients in the group who gave a history of having taken liver extract for a sufficient period of time to be considered. Six of the thirty-one were in relapse at the time the gastric analyses were taken. Since it has been shown that patients with a low red blood cell count have smaller amounts of gastric juice than those with a high red blood cell count, it is difficult to correlate the gastric juice findings of the patients in relapse and the maintenance dosage, therefore these patients were excluded.

It would seem that patients with larger volumes of gastric juice, after histamine stimulation, more often were able to maintain the blood at normal levels on smaller amounts of liver extract. However, all but two of the sixteen patients requiring more than the amount of liver extract derived from 300 grams of whole liver daily had one or more complications, such as moderate to advanced central nervous system involvement, generalized arteriosclerosis, infections, non-infectious complications (other than arteriosclerosis), or chronic diarrhea. Such complications, in themselves, have been shown by Beebe and Lewis (17) and by Fouts and Zerfas (18) to be present in a high percentage of patients requiring large daily amounts of potent liver extract. These facts make it difficult to correlate the gastric juice findings with the maintenance dosage of liver extract, however, it is interesting to note that, of nine patients with no complications, six had large total volumes of gastric juice and only one required more than 300 grams of liver daily, while one of the three patients having small total volumes required more than that amount of liver.

The amount of pepsin per cc. of gastric juice had no bearing on the amount of liver extract required by a patient. Since the total amount of pepsin secreted in sixty minutes varied approximately as did the volume, the total amount of pepsin bore the same relationship to maintenance dosage of liver extract as did the volume. The amount of rennin, likewise, had no relation to the maintenance dose of liver extract. The pH value, amount of total acid, amount of phosphorus, chlorides, and nitrogen had no correlation with the amount of liver extract required to maintain the blood at normal levels.

From these facts it is evident that the quantity and quality (as tested) of the gastric juice have little or no relationship to the maintenance dosage of liver extract. The only positive finding was that larger volumes and larger total amounts of pepsin have a questionable correlation with the maintenance dosage. Davies (3) found that two of his patients, who were able to maintain their blood at normal levels with very small amounts of liver extract, had large amounts of pepsin. We were not able to find appreciable amounts of pepsin or rennin in any of the forty-seven cases of pernicious anemia examined. Also, we could not find any correlation between the small variations in the amount of pepsin and the maintenance dosage of liver extract. We were unable to test the gastric juice for the presence of, or for the quantitative changes in, the amounts of intrinsic factor of Castle. If there are no qualitative or quantitative differences in the proteolytic enzymes of the small intestines of patients with pernicious anemia or in the amounts of the intrinsic factor, it is evident that the ability of such patients to absorb the active principle contained in liver extract largely governs the amount of liver extract required to maintain the blood at normal levels, when the extract is



administered orally Castle (19) has suggested that there is a defective absorption of liver extract in some patients having pernicious anemia and further suggests that the inability to absorb the active principle from food is of etiological importance in the disease

### CONCLUSIONS

1 The gastric juice of forty-seven patients with pernicious anemia and of ten normal persons, after histamine stimulation, has been studied

2 The complete dysfunction of the gastric glands in patients with pernicious anemia was demonstrated by the following facts (a) Small volumes of juice containing large amounts of mucus, (b) Absence of free hydrochloric acid, with an actual alkaline reaction, (c) Practical absence of the gastric enzymes, pepsin and rennin, (d) Low chloride concentration, (e) High nitrogen and phosphorus values, (f) No relation between total acid, pH, chlorides, nitrogen, and phosphorus as found in normal controls

3 The quality and quantity of the gastric juice of patients with pernicious anemia has little or no relationship to the maintenance dosage of liver extract

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# THE BLOOD FLOW IN THE BRAIN AND THE LEG OF MAN, AND THE CHANGES INDUCED BY ALTERATION OF BLOOD GASES<sup>1</sup>

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The solution of various neurological problems awaits an increase in our knowledge of the circulation of blood within the brain. Much has been gained by the demonstration of Forbes (1) and his co workers that the pial vessels of the cat are under both vasomotor and chemical control. These experiments consisted of actual inspection and measurement of pial vessels viewed through a window screwed in the skull, the pressure of the cerebrospinal fluid and of the blood being measured simultaneously. In particular, Wolff and Lennox (2) showed that changes in the gaseous content of the blood had a prompt and clear cut effect on the diameter of pial vessels, an effect which was independent of the systemic blood pressure. These authors observed that an increase in the carbon dioxide content of the blood was attended by an extreme dilatation of pial vessels whereas a decrease was followed by a constriction. Variations in oxygen content resulted in relatively small changes. An increase in oxygen tension produced a slight constriction and a decrease in tension a slight dilatation of the observed vessels.

There are two difficulties in the way of accepting these observations as significant of cerebral circulatory changes in man. First, experiments were conducted on cats under amytal anesthesia. Second, the vessels lying in the pial covering of the brain may act differently from vessels lying within the brain. Intracranial circulation is of such great importance to the organism and is so modified by the closed box arrangement of the skull that there is need for specific experimentation in man.

Observations concerning blood flow through the brain of unanesthetized persons seemed impossible until Myerson, Halloran and Hirsch (3) demonstrated the feasibility of obtaining blood from the internal jugular vein. By means of this procedure, we have repeated the experimental observations of Wolff and Lennox (2), using patients and judging

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<sup>1</sup> This is paper XX in the series of papers on "The Cerebral Circulation" from the Department of Neuropathology, Harvard Medical School. It is also from the Thorndike Laboratory of the Boston City Hospital. Work was done under a grant from the Harvard Epilepsy Commission.

changes in circulation by alterations in the gaseous content of blood entering and leaving the brain. In addition we compared the brain changes with those taking place in the leg.

A change in the oxygen content of venous blood during an experimental period might be indicative of alterations either in the oxygen consumption of the tissue traversed, or in the speed of the blood flowing through the tissues. The oxygen consumption of tissues is not affected by the tension of oxygen in the blood, within limits which were not overstepped in these experiments. Patients lay quietly throughout the period of observation, so that there was probably no great variation in the total metabolism. It is therefore believed that observed changes in arteriovenous difference were due to alterations in the speed of blood flow. Such change in pace might be consequent on change in heart rate and blood pressure, on variations in the number of open capillaries or on alterations in the size of arterioles. The observed changes in A-V difference did not parallel changes in systemic blood pressure or heart rate. We believe alterations in the size of the vascular bed is the correct explanation. This view is supported by animal experiments of Wolff and Lennox (2) in which pial vessels were watched under the microscope and by those of Bronk and Gesell (4) in which the volume of blood perfusing the head and leg was measured by means of thermopiles. Both these experimenters observed that blood flow through the parts studied did not depend on blood pressure. We believe that decreases in A-V difference (in which venous blood becomes more arterial-like) are due in our experiments to a dilatation of the arteriolar bed, leading to an increased speed and volume of blood flow. Increases in A-V differences (in which venous blood becomes more venous) are due to a constriction of the arteriolar bed with a slowing of the blood stream.

#### MATERIAL AND METHODS

The subjects used in this study were neurological patients at the Boston City Hospital, for the most part epileptics. None had serious cardiac or pulmonary defect. Observations were made only with those patients who expressed a willingness to cooperate in observations designed to increase knowledge concerning their intracranial circulation.

Observations were made in the morning, patients having fasted since the previous evening and having lain quietly for at least half an hour. Blood was taken without stasis (after anesthetization of the overlying skin) under oil and was immediately chilled and analyzed in the portable Van Slyke apparatus (5). Duplicate measurements were made and differed no more than one-tenth of a volume per cent. Arterial blood was taken from a brachial, radial or femoral artery. Blood from the internal jugular vein was secured by the method described by Myerson and associates (3), except that a small needle ( $1\frac{1}{2}$  inch 20 gauge) was used and the head was kept in a comfortable position in a line horizontal with the rest of the body.

In previous observations with the same procedure, such as those with histamine by Weiss and Lennox (6), a major difficulty had been the simultaneous

taking of blood from the various vessels. In order to meet this difficulty, as well as the objection of repeated jugular punctures, we allowed the needles to remain *in situ* in the internal jugular and femoral veins during the minutes intervening between the drawing of the preliminary and the experimental bloods. A stylette was inserted in the needle to prevent leakage and clotting. Blood was drawn simultaneously from the two veins, followed by puncture of the artery.

For purpose of comparison with the brain, a leg rather than an arm was used. Blood from the femoral vein has passed through a relatively large proportion of muscle and the circulation of the leg is not so readily affected by changes in temperature.

In order to alter the gaseous content of the blood, variations in the composition of the alveolar air were induced. The procedures used will be described later. Because there was considerable variation in individual cases, observations of each experimental procedure were made from three to ten times. Individual measurements in each of the 50 experiments are shown in the accompanying Table I.

## RESULTS

In order to distinguish the effects due to oxygen from those due to carbon dioxide, it was necessary to cover all the possible combinations of these gases. Consequently observations were separated into nine groups, with reference to whether the tension of oxygen or of carbon dioxide was increased, normal or decreased. A first additional condition was the normal control.

### *1 Normal carbon dioxide—normal oxygen*

It is important to know the amount of variation in the concentration of the gases in blood drawn from vessels at intervals. In the control experiments, 15–20 minutes elapsed between the first and second blood samples. The average differences between successive samples (which are slight) are shown in Figure 1.

### *2 Increased carbon dioxide and oxygen*

In five instances, patients breathed a mixture of 90 per cent oxygen and 10 per cent carbon dioxide. Interest attaches to these results because of the wide clinical use of a mixture of oxygen and carbon dioxide for the treatment of asphyxia, carbon monoxide poisoning and pneumonia. The gas was administered from a compressed air tank by means of a face mask. Some patients did not receive the full concentration because such violent hyperpnea was produced that dilution of the gas with air was necessary. The mixture was breathed for 5–10 minutes before blood samples were taken.

As a result of breathing this mixture the carbon dioxide content of arterial blood was increased by 3.7 volumes per cent, and the oxygen content by 0.9 volume per cent (Figure 2). The increase in the oxygen content was possible because two of the patients had an initially low arterial oxygen saturation.

TABLE I  
Gaseous content of blood before and during the breathing of various gas mixtures

Procedure and composition of inspired gas	Subject	Arterial blood before —— Per cent saturation O <sub>2</sub>	Oxygen content						Carbon dioxide content					
			Artery		Internal jugular vein		Femoral vein		Artery		Internal jugular vein		Femoral vein	
			Before	During	Before	During	Before	During	Before	During	Before	During	Before	During
			Volumes per cent											
1 Normal control —— Breathing room air  Average 4 cases	Bri (1)	94.2	18.8	12.7	12.7	14.3	14.3	14.3	53.1	59.8	59.7	57.1	57.1	
	Bri (2)	94.2	18.8	12.7	12.8	14.3	14.3	14.3	53.1	59.8	59.4	57.1	57.0	
	Ste	94.0	14.2	8.2	8.4	10.4	9.5	48.5	48.5	54.2	54.0	52.1	53.0	
	Mit	95.4	16.8	11.1	10.9	11.8	13.2	51.4	49.2	56.2	56.2	53.6	52.9	
2 Increased CO <sub>2</sub> Increased O <sub>2</sub> —— Breathing 10 per cent CO <sub>2</sub> to 90 per cent O <sub>2</sub>  Average 5 cases	Bla	94.9	17.2	11.2	11.2	12.7	12.8	51.5	51.0	57.5	57.3	55.0	55.0	
	Pre	96.0	16.9	17.6	11.1	15.4	13.9	50.0	53.9	55.2	57.5	52.3	54.7	
	Tro	95.0	18.6	19.6	12.0	18.5	16.1	55.7	55.1	61.4	62.9	57.0	58.9	
	Sto	99.0	18.3	18.3	11.8	16.3	11.5	48.2	55.1	54.7	57.7	53.3	55.9	
3 Increased CO <sub>2</sub> Normal O <sub>2</sub> —— Breathing 4 to 8 per cent CO <sub>2</sub> in room air  Average 4 cases	Ser	93.0	21.3	22.5	13.0	18.1	15.4	49.2	50.9	57.1	57.1	53.9	54.8	
		89.0	18.0	19.9	11.6	15.2	14.5	47.7	53.8	54.5	56.5	54.6	55.7	
		94.4	18.6	19.5	11.9	16.7	13.6	50.0	53.7	56.5	58.3	54.1	56.0	
	Fur	94.0	19.2	19.7	12.2	17.2	13.8	46.6	49.3	54.0	53.7	51.4	50.8	
Breathing 4 to 8 per cent CO <sub>2</sub> in room air  Average 4 cases	Sra	93.6	20.4	21.0	13.2	15.5	16.3	50.0	48.8	56.8	53.6	52.9	52.2	
	D'Amo	92.0	17.6	18.9	11.6	13.2	17.0	46.6	49.2	55.1	54.2	50.0	50.8	
	Fak	91.7	19.0	19.3	12.5	14.6	14.4	49.9	49.4	56.0	54.7	51.5	52.5	
		92.8	19.1	19.7	12.4	15.1	15.9	48.2	49.2	55.5	54.1	51.5	51.6	

TABLE I (continued)

Procedure and composition of inspired gas	Subject	Arterial blood before —— Per cent saturation O <sub>2</sub>	Oxygen content						Carbon dioxide content									
			Artery		Internal jugular vein		Femoral vein		Artery		Internal jugular vein		Femoral vein					
			Before	During	Before	During	Before	During	Before	During	Before	During	Before	During				
			Volumes per cent						Volumes per cent									
4 Increased CO <sub>2</sub> Decreased O <sub>2</sub> —— Breathing 4 to 6 per cent CO <sub>2</sub> and 8 to 12 per cent O <sub>2</sub> Average 4 cases	Tru	89.4	17.2	15.8	11.0	10.9	13.6	12.0	47.8	50.7	55.5	55.0	52.8	53.8	55.5	55.0	52.8	53.8
	Phe.	95.2	19.4	18.6	12.0	16.1	15.6	15.4	46.0	49.4	52.9	51.5	48.2	49.4	52.9	51.5	48.2	49.4
	Fak.	91.7	19.0	15.4	12.5	13.1	17.5	12.9	49.9	51.4	56.0	54.5	51.5	53.1	56.0	54.5	51.5	53.1
	Ing	96.4	19.7	16.1	10.5	9.9	15.5	13.8	46.1	45.0	54.0	51.0	48.4	48.6	54.0	51.0	48.4	48.6
5 Normal CO <sub>2</sub> to increased O <sub>2</sub> —— Breathing pure O <sub>2</sub> Average 10 cases		93.2	18.8	16.5	11.5	12.5	15.6	13.3	47.7	49.1	54.6	53.0	50.2	51.0	54.6	53.0	50.2	51.0
	Hen	92.0	17.7	19.6	11.1	11.2	11.8	18.8	47.4	43.0	51.5	53.7	50.6	46.0	51.5	53.7	50.6	46.0
	Sym	94.0	17.8	18.4	10.3	11.5	12.2	13.6	43.6	42.9	49.3	48.6	46.6	45.4	49.3	48.6	46.6	45.4
	McD	93.0	17.7	19.1	13.1	13.5	15.6	17.4	50.7	50.0	56.2	56.2	53.0	53.3	56.2	56.2	53.0	53.3
	Mor	92.0	18.0	19.6	11.2	13.3	12.8	15.0	48.2	47.6	54.4	53.4	53.1	50.9	54.4	53.4	53.1	50.9
	Shu	96.0	18.2	18.3	10.4	10.4	8.5	8.7	48.0	46.9	55.1	54.7	54.4	52.6	55.1	54.7	54.4	52.6
	Hol	90.0	18.0	19.7	11.6	12.4	13.6	15.4	47.0	44.6	53.6	53.2	49.6	49.0	53.6	53.2	49.6	49.0
	Cla	93.0	11.7	12.4	6.4	6.5	8.4	9.8	48.1	47.3	52.8	53.7	49.9	49.1	52.8	53.7	49.9	49.1
	Lev	90.0	17.1	18.6	11.1	13.5	13.0	15.9	53.7	53.7	59.9	58.9	57.7	55.9	59.9	58.9	57.7	55.9
	Col	91.0	19.9	21.8	14.8	16.4	15.3	17.1	46.5	46.3	51.8	51.9	49.3	49.8	51.8	51.9	49.3	49.8
	Cla	92.0	14.8	15.8	9.8	10.5	10.5	10.2	49.4	48.3	54.6	54.5	52.8	52.6	54.6	54.5	52.8	52.6
	Average 10 cases		92.3	17.1	18.3	11.0	11.9	12.2	14.2	47.3	47.1	53.9	53.9	51.7	50.5	53.9	53.9	51.7





TABLE I (continued)

Procedure and composition of inspired gas	Subject	Arterial blood before —— Per cent saturation O <sub>2</sub>	Oxygen content						Carbon dioxide content					
			Artery		Internal jugular vein		Femoral vein		Artery		Internal jugular vein		Femoral vein	
			Before	During	Before	During	Before	During	Before	During	Before	During	Before	During
			Volumes per cent											
Decreased CO <sub>2</sub> Normal O <sub>2</sub> —— Hyperpnea in room air	Cri	96.4	17.9	18.1	10.4	7.9	9.7	13.9	51.7	37.2	56.9	49.7	57.4	52.8
	Alt	93.0	17.0	17.4	9.4	5.9	16.1	16.3	46.2	38.4	54.4	52.4	47.5	47.8
	For	96.0	18.8	18.9	11.7	7.3	15.9	16.2	47.8	36.7	53.8	50.3	48.8	43.6
	Twa	94.7	16.2	16.9	11.0	6.5	10.9	13.5	54.7	44.2	58.5	55.0	57.8	52.8
Average 4 cases		95.0	17.5	17.8	10.6	6.9	13.2	15.0	50.1	39.1	55.9	50.1	52.9	48.5
Decreased CO <sub>2</sub> Decreased O <sub>2</sub> —— Hyperpnea in air containing 6 to 10 per cent O <sub>2</sub>	For	94.0	19.2	17.5	12.2	8.2	13.8	9.7	46.6	32.6	54.0	46.7	51.4	45.9
	Pas I	91.1	19.2	19.9	14.3	9.9	17.6	14.3	48.9	32.2	55.8	45.4	51.6	40.9
	Pas. II	94.8	19.0	15.9	11.1	6.4	16.6	14.4	48.0	37.7	56.0	47.7	50.4	40.6
	Pas III	94.8	19.0	16.2	11.1	8.6	16.6	12.7	48.0	32.5	56.0	42.3	50.4	39.4
Average 5 cases	Ing	96.4	19.7	19.0	10.5	6.8	15.5	14.3	46.1	34.6	54.0	46.9	48.4	42.6
Average 5 cases		94.2	19.2	17.7	11.9	8.0	16.0	13.1	47.5	33.9	55.2	45.8	50.6	41.9

The effect on the intracranial circulation was striking. In all cases there was a great decrease in the A-V difference, blood leaving the brain being nearly arterial, as regards its oxygen content. The average A-V difference was decreased from 6.7 volumes per cent to 2.8 volumes per cent, a decrease of 58 per cent. This indicated a vast acceleration of blood flow through the brain, a result which is in agreement with the animal experiments of Wolff and Lennox, which demonstrated a dilatation of pial vessels and an increase in spinal fluid pressure when cats breathed a

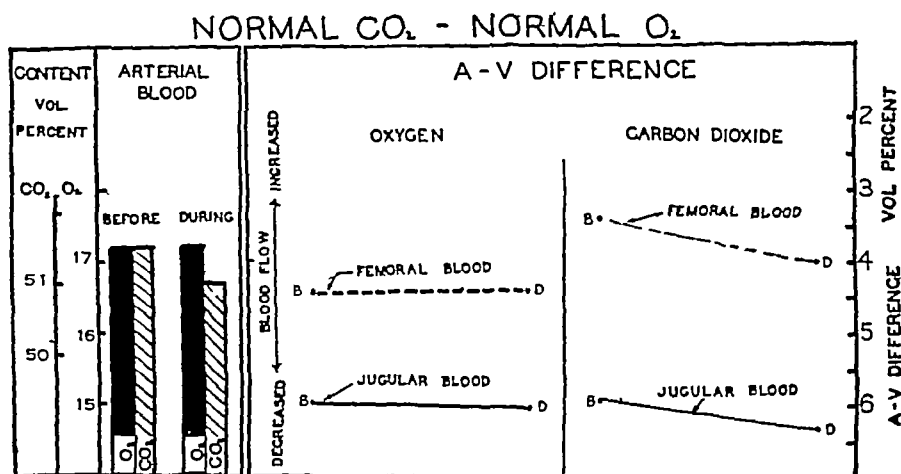


FIG 1 NORMAL CONTROL GROUP, SUBJECTS BREATHING ROOM AIR

In this and all figures except the last, the columns in the left hand portion represent the oxygen and the carbon dioxide content of arterial blood before and during the experimental period. The curves in the right hand portion represent the alterations in the gaseous content of the blood during its passage through the tissues, i.e. the A-V difference before (B) and during (D) the procedure. These changes refer to both oxygen (heavy lines) and carbon dioxide (lighter lines). The A-V differences for the leg are represented by a dashed line and for the brain by a solid line. The steepness of the slant indicates the extent of the change. A line slanting downward from left to right means that there was increased A-V difference during the experimental procedure and therefore a decreased speed of blood flow, due probably to a constriction of arterioles. An upward slant of the line represents the opposite condition. The various figures represent the average measurements of from three to 10 experiments.

similar mixture. There is agreement also with the human experiments of Cobb and Fremont-Smith (7) who noted that the color of retinal veins approached that of arteries, when subjects breathed a mixture of oxygen and carbon dioxide.

In surprising contrast to the behavior of cerebral vessels, the average A-V difference in the leg changed almost not at all (from 5.0 to 5.1 volumes per cent), an increase of two per cent. Individual results varied widely. This failure of the circulation in the leg to show changes similar to those in the brain is in keeping with the lack of a consistent rise or fall

in blood pressure. If vessels all over the body were to dilate in response to increased arterial carbon dioxide as strongly as do the cerebral vessels, there would be a dramatic fall of blood pressure.

### 3 Increased carbon dioxide—normal oxygen

In these four experiments, a Tissot tank was filled with atmospheric air to which had been added sufficient carbon dioxide to make from four to eight per cent by volume, a concentration sufficient to induce intense hyperpnea, although as compared with the previous group of experiments, the gaseous content of the arterial blood was but little increased. There was an average increase of one volume per cent in the carbon dioxide and

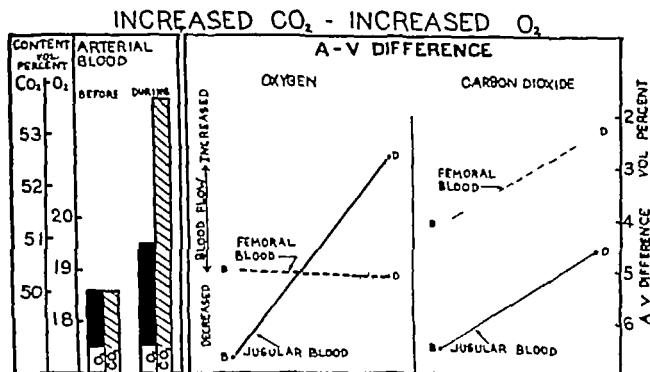


FIG 2 AVERAGE RESULTS IN A GROUP OF FIVE PATIENTS BREATHING AIR CONTAINING APPROXIMATELY 10 PER CENT CARBON DIOXIDE AND 90 PER CENT OXYGEN

The speed of blood flow through the brain is greatly increased and through the leg is not affected

0.6 volume per cent in the oxygen content of the blood (Figure 3). Venous blood drawn after an interval of five or ten minutes showed that the A-V difference was decreased in the head by 31 per cent and was increased in the leg by 50 per cent, i.e. cerebral vessels had dilated and leg vessels had constricted. In both this and the preceding group, the results in each experiment were more consistent for the head than for the leg. Of the nine experiments, changes in A-V difference in blood traversing the brain were all in the same direction, whereas in the leg, three were against the trend. The observed changes are in agreement with the animal experiments of Bronk and Gesell (4) who measured blood flow in the carotid and femoral arteries of dogs, and found the flow was increased in the carotid and decreased in the femoral artery when the animal breathed air rich in carbon dioxide.

#### 4 Increased carbon dioxide—decreased oxygen

In four instances, subjects breathed air from a Tissot tank to which both carbon dioxide and nitrogen had been added, to make a mixture containing from 4 to 6 per cent by volume of carbon dioxide and from 8 to 12 per cent by volume of oxygen. The average effect was to reduce the oxygen content of the arterial blood by 2.3 volumes per cent and to increase the carbon dioxide content by 1.4 volumes per cent (Figure 4). As in the two previous groups of experiments there was a great increase in the speed of blood flowing through the brain (a reduction in the A-V difference of 7.3 volumes per cent, or 45 per cent) whereas the A-V difference for the leg did not change.

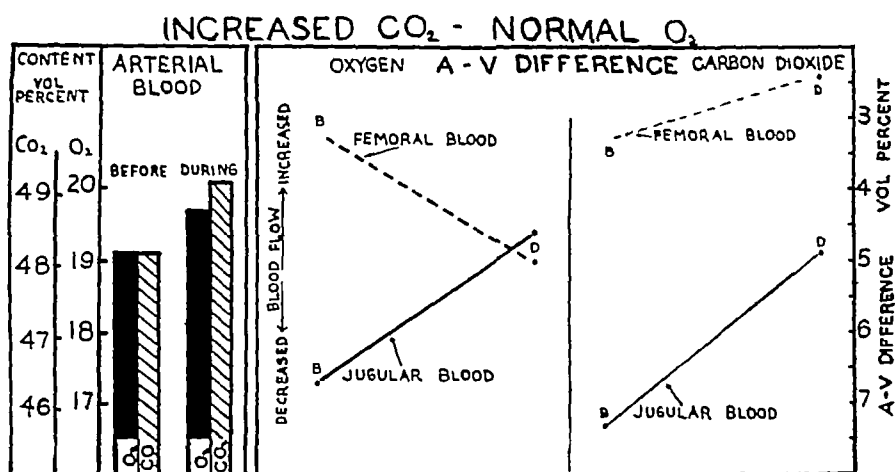


FIG 3 AVERAGE RESULTS IN A GROUP OF FOUR SUBJECTS BREATHING ROOM AIR TO WHICH HAD BEEN ADDED FROM FOUR TO EIGHT PER CENT BY VOLUME OF CARBON DIOXIDE

The speed of blood flow through the brain was greatly increased and through the leg decreased.

In each of the foregoing experiments thirteen in all, in which carbon dioxide tension of arterial blood was increased, there was a consistent and striking decrease in the A-V difference for the brain. The average decrease was 21 per cent for each volume per cent increase in the carbon dioxide content of arterial blood. In the individual cases, however, the degree of dilatation of cerebral vessels (as judged by decreases in A-V differences) was not proportional to the observed changes in the carbon dioxide content. As regards the leg, changes in A-V differences were not consistent. In eight instances there was an increase and in five a decrease.

#### 5 Normal carbon dioxide—increased oxygen

In ten experiments, subjects were attached to a Collins-Roth metabolism apparatus and breathed pure oxygen for a period of from 10 to 15

minutes While breathing oxygen the carbon dioxide content of the arterial blood did not change appreciably but the average oxygen content increased by 1.2 volumes per cent (Figure 5) This was possible because the average arterial blood was but 92.3 per cent saturated This condition of mild anoxemia in epileptic patients has been reported by Lennox (8) In contrast with the previous groups of experiments, changes in blood flow were slight and not consistent For the brain, the A-V difference was increased in six instances and decreased in four, the average change being an increase of five per cent For the leg, the A-V difference was increased in two instances and decreased in eight, the average change being a decrease of 16 per cent The results therefore are indecisive, there

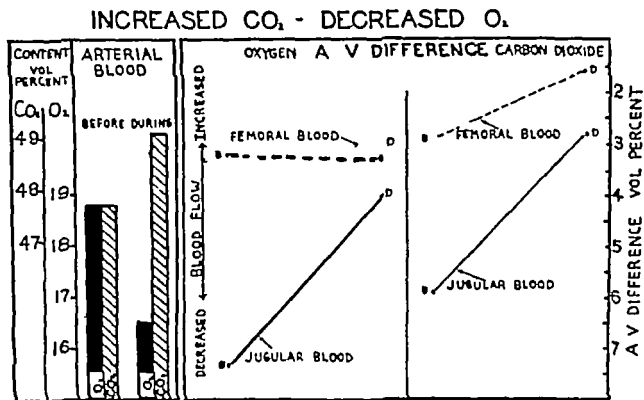


FIG 4 AVERAGE RESULTS IN A GROUP OF FOUR SUBJECTS WHO BREATHED AIR CONTAINING FROM FOUR TO SIX PER CENT BY VOLUME OF CARBON DIOXIDE AND FROM 8 TO 12 PER CENT OF OXYGEN

The speed of blood flow through the brain was very greatly increased whereas the flow through the leg was not affected

being evidence of a slight decrease in the speed of blood flow through the brain, and more consistent evidence of an increased flow through the leg So many experiments were conducted because of the lack of consistency in results and because changes in circulation due to the breathing of oxygen have received scant attention in medical literature In dog experiments Bernthal, Bronk, Cordero and Gesell (9) obtained evidence of a decreased blood flow in both the carotid and the femoral arteries Our results for the circulation in the leg, therefore, are not in agreement with theirs The difference may be due to the fact that presumably the anesthetized animals had a greater initial anoxemia than the human subjects

### 6 Normal carbon dioxide—slightly decreased oxygen

In four experiments subjects breathed from a Tissot tank to which atmospheric air and nitrogen had been added. Individuals breathing the same gas mixture showed large differences in the degree of anoxemia. Therefore, separation of cases was based on the actual degree of anoxemia present in arterial blood and not on the mixture breathed. The average reduction in arterial oxygen in the four subjects of this group was one volume per cent making the arterial blood 87 per cent saturated. Because of the hyperpnea induced by the diminished oxygen supply, the average carbon dioxide content of the arterial blood was decreased by 1.9 volumes per cent (Figure 6). In three of the four cases there was a sharp increase

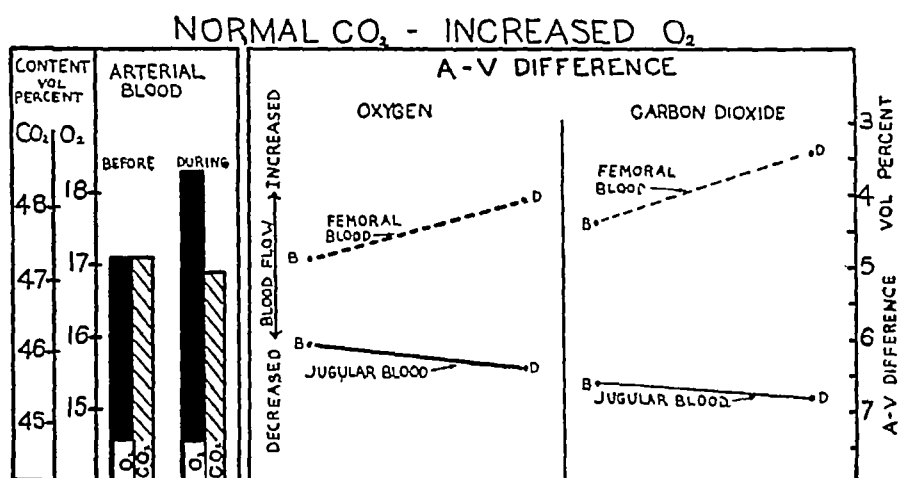


FIG 5 AVERAGE RESULTS IN A GROUP OF TEN SUBJECTS WHO BREATHED PURE OXYGEN

The speed of blood flow through the brain was slightly decreased, and through the leg slightly increased.

in the A-V difference for the brain. The average increase for the four cases was 30 per cent. For the leg, the A-V difference was increased in two and decreased in two experiments, the average change being an increase of 5 per cent. The decreased speed of blood flow through the brain, in the light of evidence from other groups of experiments, was presumably caused by the reduction of carbon dioxide, rather than the reduction of oxygen in the arterial blood.

### 7 Normal carbon dioxide—greatly decreased oxygen

In these seven cases, a much greater degree of anoxemia was induced, the average oxygen content of arterial blood being reduced by 5.9 volumes per cent (from an average saturation of 93.4 per cent to 87.5 per cent). In spite of this anoxemia, the average carbon dioxide content of the arterial blood fell but three volumes per cent (Figure 7).

In all seven instances there was a decrease in the difference in oxygen content between arterial and internal jugular blood, the average decrease being 31 per cent. In five instances there was likewise a great decrease in the difference between arterial and femoral vein blood (the average decrease for the seven cases being 48 per cent). Therefore, the vessels of both the brain and the leg underwent dilatation. This is in agreement with observations in the dog by Gesell and his associates. If all the blood vessels of the body dilated to this extent, there would be a fall in systemic blood pressure. We were not able to record the pulse and the blood pressure readings in all these cases, but in those in which we did, there was an increase in heart rate and a fall in blood pressure. According to Marshall (10) in conditions of anoxemia, cardiac output is increased only when the concentration of oxygen in the inspired air is less than 11

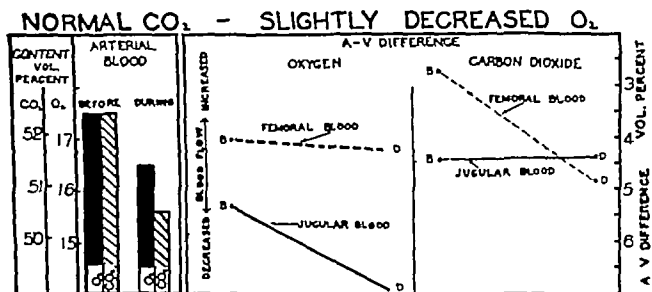


FIG 6 AVERAGE RESULTS IN A GROUP OF FOUR SUBJECTS WHO BREATHED AIR CONTAINING FROM 6 TO 12 PER CENT BY VOLUME OF OXYGEN AND WHOSE ARTERIAL OXYGEN SATURATION DECREASED FROM A PRELIMINARY VALUE OF 92.3 PER CENT TO 87 PER CENT

There was a moderate decrease in the speed of blood flow through the brain and a very slight decrease in the leg, due probably to the diminution in the tension of carbon dioxide in arterial blood.

per cent. The fact that there was decreased cerebral blood flow with slight anoxemia (Group 6) and increased flow with extreme anoxemia (Group 7) is probably explainable in this manner. The decreased carbon dioxide due to dyspnea exerted its constricting effect when anoxemia was trivial, but an even greater carbon dioxide loss could not prevent dilatation when anoxemia was extreme.

#### 8 Decreased carbon dioxide—increased oxygen

In three experiments, subjects while connected with a basal metabolism machine and breathing pure oxygen, performed voluntary hyperpnea. Arterial blood drawn after five or ten minutes revealed an average increase of 5 volume per cent in oxygen content and a decrease of 10 volumes per cent in carbon dioxide content (Figure 8).



In each instance there was an increase in the A-V difference for the brain, the average increase being 47 per cent and a decrease for the leg, the average being 19 per cent. These changes indicate a decreased speed of blood flow through the brain and an increased flow through the leg.

### 9 Decreased carbon dioxide—normal oxygen

In this group of four experiments, the subjects performed over-ventilation in room air, a procedure which increased the average oxygen content of arterial blood 3 volume per cent and decreased its carbon

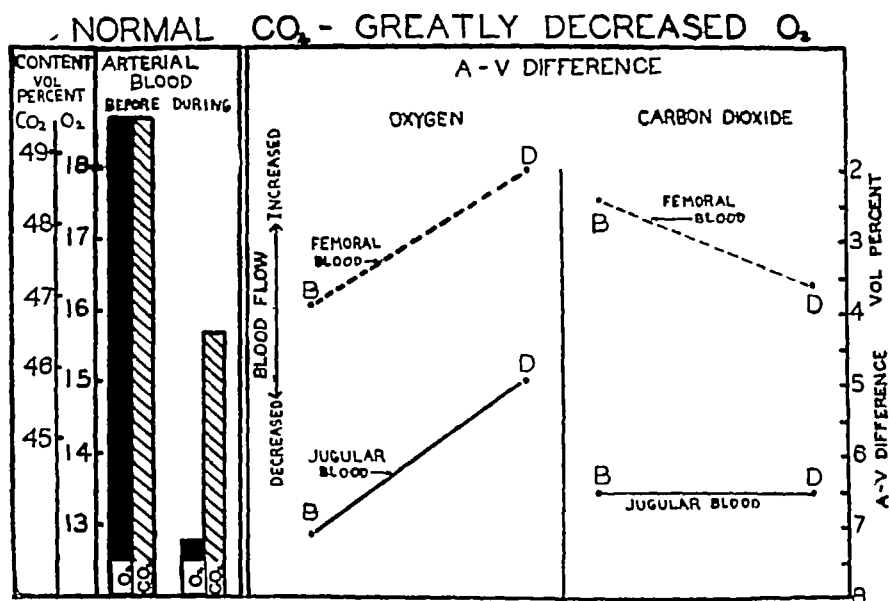


FIG 7 AVERAGE RESULTS IN A GROUP OF SEVEN PATIENTS WHO BREATHED AIR CONTAINING FROM 6 TO 8 PER CENT BY VOLUME OF OXYGEN, AND WHOSE ARTERIAL OXYGEN SATURATION DECREASED FROM A PRELIMINARY VALUE OF 93.4 PER CENT TO 64 PER CENT

The flow of blood through both the brain and the leg was greatly accelerated

dioxide content by 11 volumes per cent. Results were not greatly different from those in the preceding group. The effect on blood flow was in the same direction though more distinct. For the brain there was an increase in the A-V difference in all cases, the average increase being 58 per cent. For the leg there was a decrease in the A-V difference in three of the four cases, the average decrease for all being 35 per cent (Figure 9). The decrease in cerebral blood flow accompanying hyperpnea (per unit change in carbon dioxide content of arterial blood) is only one sixth as great as the increase which accompanies the breathing of carbon dioxide.

10 *Decreased carbon dioxide—decreased oxygen*

In three experiments, subjects breathed a mixture of air and nitrogen (the oxygen forming from 6 to 10 per cent by volume of the mixture) from a Tissot tank, at the same time performing voluntary hyperpnea. In two experiments subjects were attached to a basal metabolism apparatus, the bell of which had been filled with room air. In this case anoxemia developed more slowly.

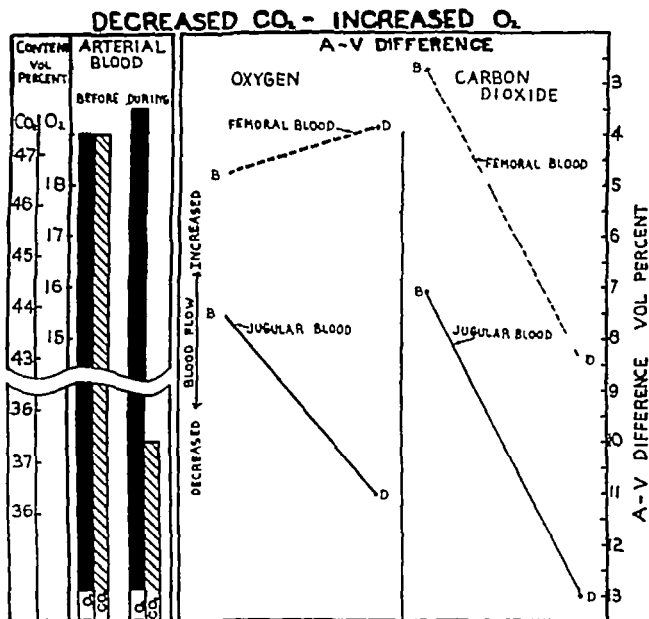


FIG 8 AVERAGE RESULTS IN A GROUP OF THREE SUBJECTS WHO PERFORMED HYPERPNEA WHILE BREATHING PURE OXYGEN

The speed of blood flow through the brain was greatly decreased and through the leg was slightly increased.

Although air mixtures were practically the same as in the experiments under Group 7, the anoxemia produced was not as great when subjects breathed deeply, as when they breathed naturally. The percentage saturation of arterial blood was reduced by only 6.6 per cent, against a reduction of 29.4 per cent when hyperpnea was not performed. Schneider (11) has stated that under conditions of reduced atmospheric pressure subjects are more comfortable if they breathe deeply. In the five experiments the average decrease in the oxygen content of arterial blood was 1.5 and of the carbon dioxide content 13.6 volumes per cent.

In four instances, the A-V difference was increased both for the brain and the leg, in one it was decreased for both. For the five experiments, the average increase for the brain was 33 per cent and for the leg 44 per cent (Figure 10)

Although overbreathing in oxygen poor air resulted in a greater decrease in the carbon dioxide content of arterial blood than overbreathing in room air, the diminution in blood flow through the brain was less. Presumably this was due to the counteracting effect of the anoxemia which in itself would cause an increase in blood flow.

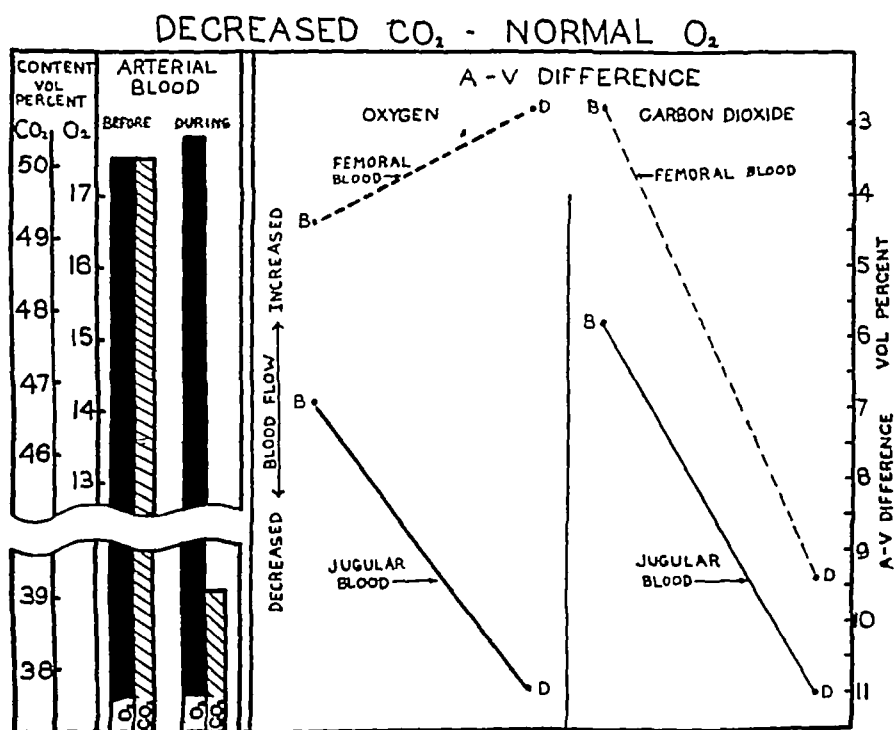


FIG 9 AVERAGE RESULTS IN A GROUP OF FOUR SUBJECTS WHO PERFORMED HYPERPNEA WHILE BREATHING ROOM AIR

The speed of blood flow through the brain was greatly decreased and through the leg it was slightly increased.

The decrease in the flow of blood through the leg is not so easy to explain, for in Groups 7, 8 and 9, in which there had been a decrease either in the carbon dioxide or in the oxygen content of the arterial blood, the leg flow had increased. Throughout this investigation, the blood flow through the leg was more variable than the flow through the brain.

#### *A-V differences in carbon dioxide content*

In the presentation of material thus far, we have dealt only with changes in oxygen tension of the blood. In Figures 1-10 the A-V dif-

ferences in carbon dioxide content are shown in the right hand portion. One would expect that curves representing changes in oxygen and in carbon dioxide content would slope in opposite directions, e.g. the A-V difference for carbon dioxide should increase as that for oxygen decreases. Of the nine experimental groups the slopes are opposite in five for the leg and in only one for the brain. As we have pointed out (12) the respiratory quotient (the increase in carbon dioxide of venous blood, divided by its decrease in oxygen with reference to the arterial blood) of the brain in

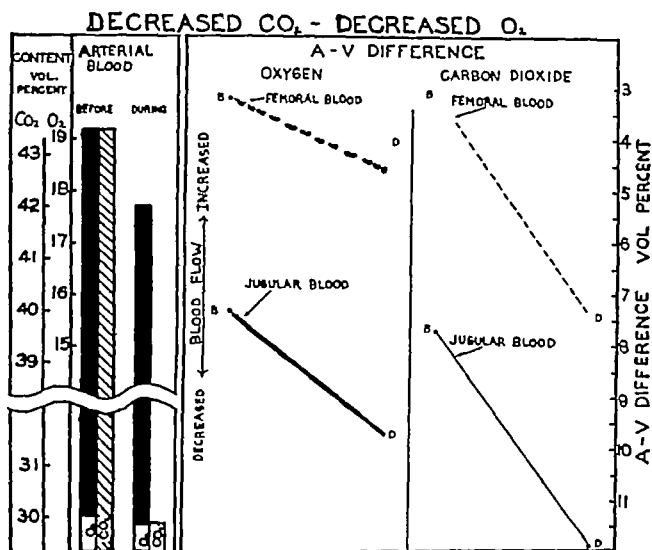


FIG 10 AVERAGE RESULTS IN A GROUP OF FIVE SUBJECTS WHO PERFORMED HYPERPNEA WHILE BREATHING AIR CONTAINING FROM 6 TO 10 PER CENT OF OXYGEN

The speed of blood flow through both the brain and the leg was decreased

these unanesthetized subjects is much higher than that of the leg. Under conditions in which the composition of the alveolar air is artificially altered the respiratory quotient is greatly distorted. In the low oxygen experiments one factor in such distortion is the various degrees of absorption of carbon dioxide by the tissues. In the ten experiments in which patients quietly breathed pure oxygen (Group 5) the average respiratory quotients before and after the procedure were approximately the same. In the seven experiments in which marked anoxemia was produced (Group 7), the quotients during the breathing were above unity, 1.32 for the brain and 1.80 for the leg, against preliminary averages of .91

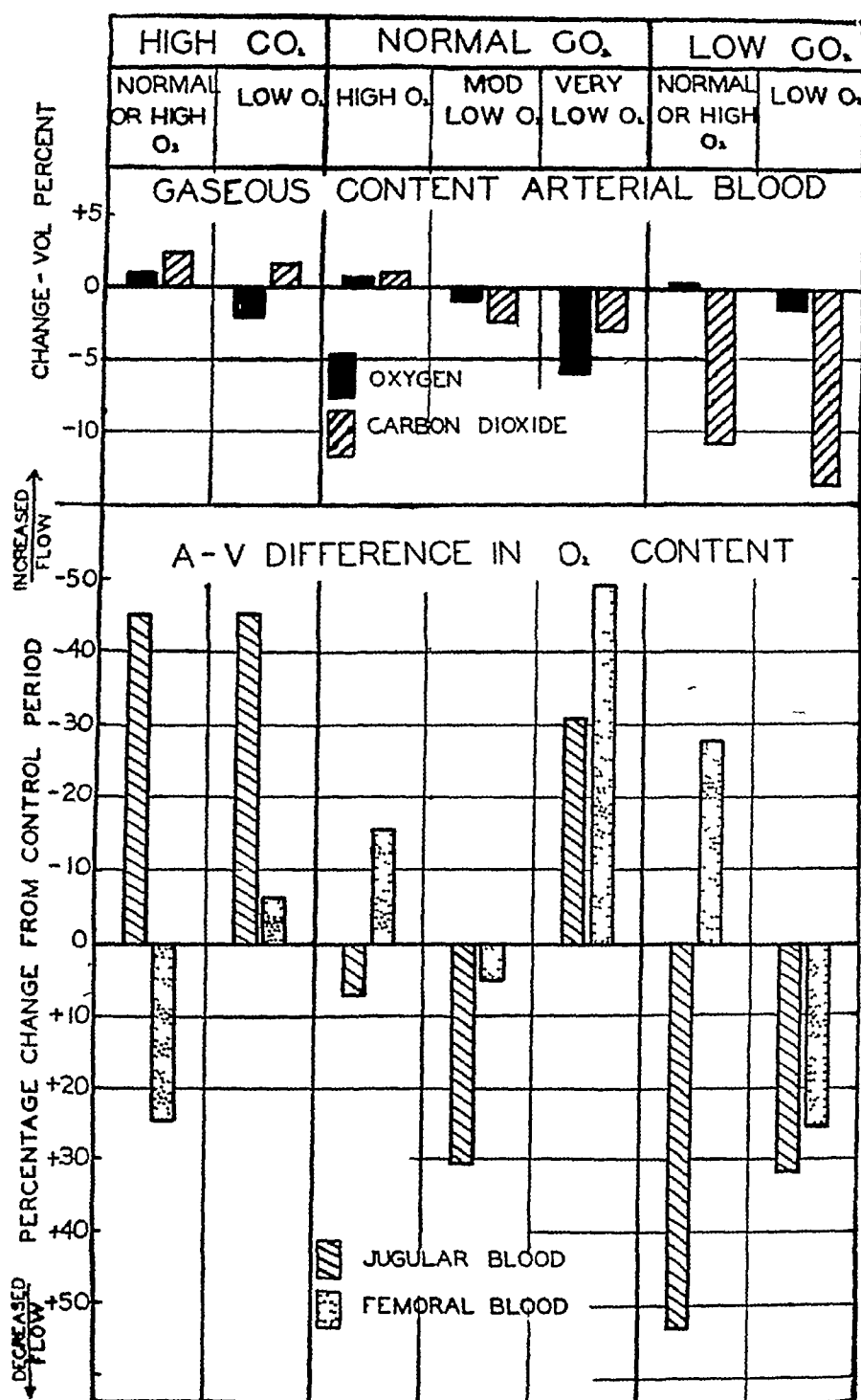


FIG 11 THE AVERAGE RESULTS IN SEVEN GROUPS OF EXPERIMENTS WITH 50 SUBJECTS

The upper portion of the figure represents the changes in the gaseous content of arterial blood which followed alteration of the alveolar air, induced by breathing various mixtures of carbon dioxide and oxygen. Projection of

and 62 respectively. This excess of carbon dioxide in the venous blood suggests that under conditions of anoxemia carbon dioxide is not readily absorbed by the tissues, and more carbon dioxide is absorbed by the brain than by muscle. McGinty (13) found that tissues of living frogs do not absorb carbon dioxide as readily when the animals are kept in a low oxygen atmosphere and that the brain absorbed more than muscles. If the differences in respiratory quotient between the brain and the leg had been due to differences in the distribution of capillaries in the brain and leg (a possibility suggested by Myerson, Loman, Edwards and Dill (14) for similar respiratory quotients in their cases) one would expect distinct differences in blood flow. On the contrary the increase in the speed of blood flow in Group 7 was practically the same in the brain as in the leg.

#### DISCUSSION

The detailed changes in blood flow which have been enumerated are confusing. In order to contrast more clearly the average changes in each group, we calculated the percentage change in the A-V oxygen difference for the experimental as contrasted with the control period. These results are depicted in Figure 11.

At the top of the chart is shown the average change from the pre-experimental sample as regards the gaseous content of arterial blood. The changes in the composition of blood flowing through the brain are clear cut and consistent. An increase in the carbon dioxide content of the arterial blood causes an increase of more than 40 per cent in blood flow, as measured by alterations in the oxygen content of venous blood. On the other hand, a similar decrease in the carbon dioxide of arterial blood results in a decrease in blood flow only a third or a fourth as great. Wolff and Lennox observed this also when watching pial blood vessels.

The effect of oxygen is less. An increase of oxygen in arterial blood causes a slight decrease in cerebral flow, a well marked anoxemia an increase. When the degree of anoxemia is not great, any change in the carbon dioxide tension of blood overshadows it. However, anoxemia partially neutralizes the constricting effect of diminished carbon dioxide. Also, anoxemia augments the dilator effect of carbon dioxide and excess oxygen augments the constrictor effect of decreased carbon dioxide.

These conclusions are in entire agreement with the window observations of Wolff and Lennox. Dilatation of pial vessels of cats occurred

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columns above or below the base line indicates that the gaseous content had been increased or decreased. The solid columns indicate oxygen content, the double hatched columns carbon dioxide content. The lower portion of the figure represents changes which took place in the A-V difference of blood oxygen. Projection of columns above the base line indicates that the A-V difference had decreased and the speed of blood flow had increased. Projection below the line indicates the opposite. The single hatched columns represent the brain and the dotted columns the leg.

under the same circumstances that cause decrease in the difference in the blood gases entering and leaving the brain. The effect of changes in oxygen tension of arterial blood was more clear cut in the human than in the cat experiments, due presumably to the fact that the anesthetized animals had a mild degree of anoxemia.

Our observations demonstrate that the changes in the diameter of pial vessels do not indicate, as Kubie and Hetler (15) suggested, merely a local change in pial circulation, but reflect, rather, a change in circulation throughout the brain. Likewise they contradict the contention of Nicholson (16) that the increase in cerebrospinal fluid flow which accompanies breathing of carbon dioxide is not due to enlargement of the cerebral vascular bed.

Concerning the effects of altering blood gases on the circulation of the leg, the evidence is less clear cut. Given a normal or better than normal arterial oxygen tension, an increased carbon dioxide tension was associated with an increased A-V difference (decrease in blood flow). A decreased carbon dioxide tension was associated with a decreased A-V difference (increase in blood flow). This is the opposite to the condition for the brain. In the presence of a profound arterial anoxemia (the carbon dioxide tension being only slightly affected) there is a markedly increased blood flow, through both leg and brain.

In general, it may be said that circulatory responses to chemical stimuli are greater in the brain than in the leg. One reason for this is the fact, pointed out by Lennox and Leonhardt (17), that the A-V difference is greater for the brain than for the extremities. Therefore there is greater opportunity for a decreased A-V difference, in other words, for an improvement in the quality of the blood pouring through the brain. Cerebral circulatory responses are also more constant than those of the leg from time to time and from subject to subject.

These observations demonstrate that the body has a mechanism for altering the speed and the composition of the blood flowing through the brain. Either anoxemia or increase of carbon dioxide is attended by a dilatation of vessels, which provides capillaries and veins with blood that is more nearly arterial in quality. The opposite conditions result in a retardation of the blood stream. The chemical mechanism which governs these circulatory changes is probably a complicated one. In the case of the carbon dioxide, the coincident alterations in the acid base relationships of the blood are undoubtedly important. Injection of acid substances other than carbon dioxide or of alkaline substances also result in dilatation and constriction of brain vessels. Presumably the increased cerebral flow consequent on anoxemia cannot be explained on the basis of acid base changes in the blood, for according to Koehler, Brunquist and Loevenhart (18) acidosis occurs only when the anoxemia is extreme and prolonged. Whatever the exact mechanism, these adjustments in blood

flow provide brain tissue with blood which is more constant in quality than would otherwise be the case. Presumably this contributes to more effective functioning of the brain.

The fact that circulatory adjustments for the brain may be and usually are the opposite of those for the leg is of great theoretical and practical interest. This circumstance permits alteration of blood flow in the brain without change in the systemic blood pressure. It permits the brain to secure preferential treatment as regards allocation of arterial blood. In these experiments the differential action is more evident after alteration of carbon dioxide than after alteration of oxygen tension. When the carbon dioxide tension of the arterial blood was increased or reduced (and oxygen tension not reduced) the speed of blood flow in the head and leg was affected in an opposite manner. On the other hand, when anoxemia was induced (no matter what the carbon dioxide tension) the blood flow in the brain and leg increased or decreased together. These are average, not individual results.

These experiments do not shed light on the localization of the mechanism of chemical control, whether in a center in the brain or in the walls of the blood vessels. One is perplexed to explain how the same chemical stimulus produces such diverse vasomotor effects. Nor can one explain the differing reactions on the basis of the closed box arrangements of the skull. Though vessels in the brain are embedded in an incompressible medium, the alterations in calibre, as measured by A-V differences, are even greater in the brain than in the leg. Of course, one must take into account possible differences in the area of the vascular bed in relation to the volume of brain and leg tissues. According to the researches of Cobb (19) nervous tissue has fewer capillaries per square millimeter than has muscle. Again, capillaries in the two regions may respond differently to chemical stimuli, or there may be differing responses of capillaries and of arterioles. The relative scarcity of capillaries in the brain, together with the external resistance to capillary expansion, may hinder capillary stasis and hence, when arterioles dilate, result in a relatively speedy flow of blood through the brain.

Finally our observations emphasize the contention of Wolff and Lennox (2) that when the patient benefits from measures which alter the composition of alveolar air, in any assignment of credit, the accompanying changes in cerebral circulation must be taken into account.

#### SUMMARY

Fifty observations were made of changes in the speed of blood flow through the brain and the leg of man. These changes were in response to alteration in the composition of the alveolar air, and were judged by the alterations in the difference in the oxygen content of blood entering and leaving the brain and the leg.



Alteration in the composition of alveolar air was attended by a marked change in the speed of blood flow. With an increased tension of carbon dioxide in arterial blood, the speed of flow through the brain was increased and through the leg was decreased. With a decreased tension of carbon dioxide the flow through the brain was decreased and through the leg increased.

Alteration of the oxygen content of arterial blood produced less pronounced results. Increase in the oxygen content resulted in a slight decrease in speed of blood flow through the brain and increase through the leg. Pronounced anoxemia resulted in increased flow in both brain and leg.

Changes in speed of flow in these experiments are believed to be due to dilatation or constriction of arterioles.

An increase in the flow in the brain is more readily produced than a decrease. Alterations in the flow are more consistent in the brain than in the leg. Conclusions are based on average, not individual, results.

In conditions of anoxemia the tissues of the brain and the leg absorb less than the usual amount of carbon dioxide.

Circulatory adjustments for the brain, in response to gaseous changes in the blood, may be the opposite for those in the leg, thereby permitting the brain to receive preferential treatment as regards allocation of arterial blood.

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# THE BLOOD PICTURE IN EXOPHTHALMIC GOITRE AND ITS CHANGES RESULTING FROM IODINE AND OPERATION A STUDY BY MEANS OF THE SUPRAVITAL TECHNIQUE

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The purpose of this report is to record a series of cases of exophthalmic goitre studied intensively by the supravital technique of blood examination This method was chosen as being particularly suited to the problem since it affords a clear differentiation of the large lymphocyte, monocyte and transitional polymorphonuclear cells, a distinction which is not always possible in the ordinary type of fixed smear

## HISTORICAL

While Ciuffini (1) described relative lymphocytosis in exophthalmic goitre in 1904, this aspect of the disease did not attract universal attention until the publications of L Caro (2) (3) In the year 1907 the latter author described a case of fatal exophthalmic goitre with such atypical findings in the blood that he thought he was dealing with an associated pseudoleukemia Later (1908) he collected a series of cases showing similar blood pictures Caro was furthermore able to produce the changes in the blood of normal individuals by the oral administration of thyroid gland He showed that patients with non toxic varieties of goitre did not have any alteration in their blood picture The studies of Kocher (4) in 1908 received the attention of the entire medical profession He described a triad of blood findings in Basedow's disease which he thought could be used in the early diagnosis of the malady This triad consisted of leukopenia relative hypopolynucleosis and relative lymphocytosis It has come to bear his name as the "Kocher blood picture." Crotti (5) was able to confirm the observations of Kocher and stated that the blood findings were helpful in diagnosing the disease and in estimating its severity

Crotti (5) observed cases during the postoperative period before the introduction of iodine in the preoperative treatment We quote him directly as follows "The same day of the operation the lymphocytes diminish materially while the polynuclears increase on the following days, however, the blood formula returns to its previous pathological state, and only then improves gradually in direct proportion with the disease and usually becomes normal in the cured cases"

DeQuervain (6) stated that the above changes were not limited to exophthalmic goitre, for they occurred in other types of thyroid dyscrasia Müller (7), Bauer (8) and Lampé (9) were of the same opinion

Probably the most extensive study of the subject was that of W A Plummer (10) in 1919 He examined the blood of 578 cases of hyperthyroidism and recorded an average leukocyte count of 6,793 cells per cu mm The relative

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lymphocytosis and hypopolynucleosis, although appreciable in his figures, were not as definite as those recorded by Kocher and Crotti. He found no relationship between the blood changes and mortality in his series, and concluded that the blood picture was of little aid in diagnosis or prognosis.

Menkin (11) in 1928 studied 100 case records of hyperthyroidism, and followed the blood changes in a small series of patients. His conclusions were that relative lymphocytosis was present in hyperthyroidism, but that it was more frequent and more marked in cases with definite exophthalmos. Examination of his protocols reveals that several of his cases of "hyperthyroidism with exophthalmos" had elevated monocyte percentages as well as relative lymphocytosis, a fact which he failed to note. He also presented data to show that after operative treatment of the disease there was a reversion toward a normal blood status. However, no account was taken in his study of the effect of preoperative medication per se on the blood picture.

Most recently, Jackson (12) concluded, from a study of the blood of 600 cases of thyroid disease of various forms, that there was no basis for the Kocher triad, that iodides had no effect on the blood formula, and that there was no parallelism in the height of basal metabolic rate, the severity of the disease and the differential blood picture.

### METHOD

The method of blood examination used in this study has been described in detail by Sabin (13). It consists, in its main features, of the following steps: thin smears of non-toxic stains, neutral red and janus green, are made on clean, polished slides. Blood is received on a coverslip and allowed to spread on the thin film of stain. The coverslip is then rimmed with vaseline to prevent evaporation. The preparation is examined at body temperature in a warm box with oil immersion objective.

The appearance of the cells with this technique is such that cytoplasmic structure is more clearly made out than in fixed smear. The polymorphonuclear cells are the most conspicuous since their finely granular cytoplasm is in constant amoeboid motion. Lymphocytes are characterized by their lack of granulation, their high mitochondrial content and the paucity or complete absence of components staining with neutral red. The monocyte is motile, more so than the sluggish lymphocyte, and contains a few mitochondria. It is mainly identified by a group of granules which selectively take the neutral red stain and are arranged, for the most part, in the form of a rosette in the crescent of the large indented nucleus. Nuclear contour and consistency are readily appreciated in the supravital technique despite the fact that nuclear material does not take up the stains used. The chromatin network, fine in the monocyte and coarse in the lymphocyte, is a distinguishing feature.

### I

## BLOOD PICTURE IN EXOPHTHALMIC GOITRE

### MATERIAL

Since our preliminary study indicated that exophthalmic goitre was more apt to show an altered blood picture than other types of goitre, we

decided to study only those cases in which a definite diagnosis of exophthalmic goitre had been made by the Thyroid Clinic. Only patients who had not received iodine previous to hospitalization were included. Care was exercised to allow for the factors known to affect the blood formula. Counts were done at approximately the same hour each time for individual patients, usually between 2.30 and 4.00 P M.

We were unable to demonstrate any changes of importance in the blood picture on rest alone during the four or five day period when levels of metabolism and blood counts were being obtained. The results for thirty consecutive cases are given in Table 1.

TABLE 1

*Initial levels of basal metabolic rate and blood findings in 30 patients with exophthalmic goitre*

Patient number	Leuko-cytes	Basal metabolic rate	Neutrophils*	Lymphocytes	Monocytes	Eosinophiles	Basophiles
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	5,000	60	55	25	14	4	2
2	6,000	48	60	20	11	5	4
3	4,300	68	65	15	17	2	1
4	9,200	74	67	15	18	0	0
5	4,850	50	50	20	23	4	2
6	5,900	44	61	24	10	4	1
7	5,000	52	64	18	12	5	1
8	6,500	58	60	15	20	3	2
9	6,800	47	65	19	15	1	0
10	7,800	61	58	29	13	0	0
11	6,800	56	54	28	15	2	1
12	6,850	50	40	38	18	2	2
13	5,100	56	66	18	14	1	1
14	5,900	38	46	37	13	4	0
15	5,450	36	54	30	12	3	1
16	7,150	51	64	14	19	3	0
17	4,200	52	45	34	15	4	2
18	6,250	39	54	25	16	5	0
19	5,000	30	52	24	17	4	3
20	6,250	38	56	30	12	1	1
21	4,700	30	58	21	13	7	1
22	6,900	40	63	20	15	2	0
23	5,700	24	57	26	14	3	0
24	8,200	25	62	21	10	6	1
25	6,500	42	55	28	5	8	4
26	13,000	42	61	17	15	4	3
27	10,400	40	57	29	12	1	1
28	6,800	28	56	26	15	3	0
29	5,750	31	49	37	8	4	2
30	6,600	42	50	32	12	5	1
Average	6,495	45.1	56.9	24.5	14.1	3.3	1.2
Standard deviation of average	331	2.24	1.20	1.25	0.65	0.35	0.21

\* Includes stab forms of leukocytes

Most of the cases were of moderate severity as judged by the levels of metabolism. Twenty-three of the thirty cases fell into a group in which the basal metabolic rate varied from plus 30 to plus 59. There were three cases in which the metabolic rates were higher, and four in which they were lower.

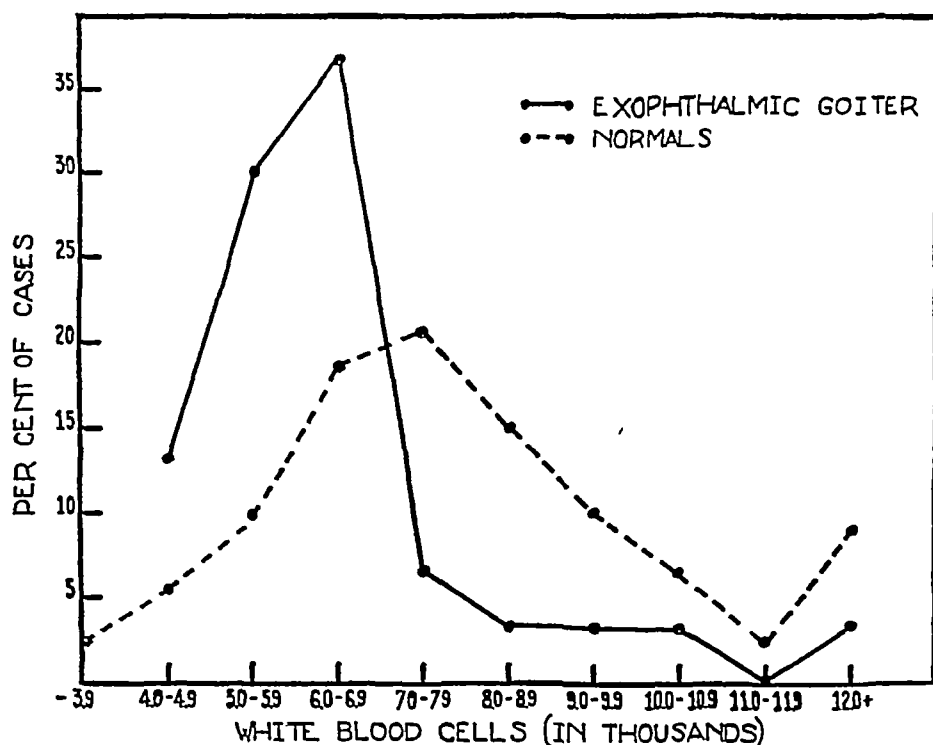


FIG 1 COMPARISON OF AVERAGE LEUKOCYTE COUNTS IN 30 CASES OF EXOPHTHALMIC GOITRE AND 310 NORMALS

#### *Total white blood cells*

Figure 1 represents the percentage distribution of the white blood cell counts of 30 exophthalmic goitre patients and of 310 normal people collected by Lerman (14). The curves demonstrate that in exophthalmic goitre there is a skewness toward the leukopenic side of the blood picture. Of the goitre patients 80 per cent had counts below 7,000 cells, whereas only 36 per cent of normals had counts below this level. This points to the existence of a definite leukopenia in the disease, a fact which is not so obvious from the average leukocyte count of the series. The latter is  $6,495 \pm 331$  cells. Compared to the normal average leukocyte count of  $7,850 \pm 132$  cells as obtained by Lerman (14), the difference is  $1,335 \pm 357$  or 3.8 times its standard deviation. It is, therefore, highly significant statistically. In this connection it is important to note that all the counts in our study were done in the afternoon and therefore represent the peak in the diurnal variation in total white blood cell

counts (15) Morning counts in several instances were recorded as low as 2,400 to 3,100 in patients who had afternoon counts of 5,000 to 6,000 cells This fact gives additional significance to the recorded difference

### *Differential blood picture*

Tables 2, 3, and 4 represent the distribution of the polymorphonuclear neutrophils, lymphocytes and monocytes respectively We have been unable to find any large series of normal differential counts made by the supravital technique Consequently we cannot make a final comparison of the above distributions with the normal The results given by Sabin

TABLE 2

*The distribution of the neutrophilic leukocyte percentages of 30 patients with exophthalmic goitre*

Neutrophils per cent	Number	Per cent
—45	2	6.6
45–48	2	6.6
49–52	3	10.0
53–56	9	30.0
57–60	6	20.0
61–64	6	20.0
65–68	2	6.6

TABLE 3

*The distribution of total lymphocyte percentages of 30 patients with exophthalmic goitre*

Lymphocytes per cent	Number	Per cent
14–17	5	16.6
18–21	8	26.6
22–25	4	13.3
26–29	6	20.0
30–33	3	10.0
34–37	3	10.0
38+	1	3.3

TABLE 4

*The distribution of monocyte percentages of 30 patients with exophthalmic goitre*

Monocytes per cent	Number	Per cent
—10	2	6.6
10–12	8	26.6
13–15	12	40.0
16–18	5	16.6
19–21	2	6.6
22+	1	3.3

(15), based on 175 counts on six normal people, may be used tentatively as the normal level for the supravital method In Table 5 these results are compared with the averages obtained in 30 patients with exophthalmic goitre We cannot compare the absolute number of cells in the same way because Sabin's data do not contain the averages of the absolute numbers However, normal averages may be obtained by multiplying



Sabin's percentages by the normal total leukocyte count given above. These normals are of limited value because their standard deviations cannot be calculated.

### *Polymorphonuclear neutrophiles*

The group distribution given in Table 2 does not show any distinct tendency to hypopolynucleosis in our series if the comparison is made with results based on the fixed smear method. However, compared to the results of Sabin (15), such a tendency is definite as revealed by the average figures given in Table 5. The difference between the normal

TABLE 5

*Comparison of the average percentages of the differential count in exophthalmic goitre and in normal people (Sabin)*

	Neutrophiles	Lymphocytes	Monocytes	Eosinophiles	Basophiles
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Exophthalmic goitre	$56.9 \pm 1.20$	$24.5 \pm 1.25$	$14.1 \pm 0.65$	$3.3 \pm 0.35$	$1.2 \pm 0.21$
Normal	$68.9 \pm 2.18$	$19.7 \pm 1.20$	$5.7 \pm 0.49$	$4.4 \pm 1.15$	$1.2 \pm 0.21$
Difference between exophthalmic goitre and normal averages	$12.0 \pm 2.49$	$4.8 \pm 1.73$	$8.4 \pm 0.81$	$1.1 \pm 1.20$	0.0

and exophthalmic goitre neutrophile counts is  $12.0 \pm 2.49$  per cent, a value of high statistical significance. Moreover, since the diurnal variation in total white blood cell count is mainly attributable to the fluctuation of the neutrophiles, the above percentage of neutrophiles in exophthalmic goitre, obtained in the afternoon, represents the peak of the curve. Consequently hypopolynucleosis is actually greater throughout the day than our results indicate. The absolute numbers of neutrophiles in exophthalmic goitre is reduced to a greater extent than the percentage because of the simultaneous reduction in total leukocyte count. Thus the average neutrophile count for the exophthalmic goitre cases is 3,696 cells as compared with the normal of 5,409 cells.

### *Lymphocytes*

The distribution of lymphocyte percentages is given in Table 3. The average percentage is  $24.5 \pm 1.25$ , which is greater than the normal by  $4.8 \pm 1.73$  per cent. The difference, though not large, indicates a significant tendency to relative lymphocytosis in exophthalmic goitre. About 68 per cent of the patients showed lymphocyte percentages above the normal average of 19.7, and 23.3 per cent showed percentages of 30 or more. The absolute number of lymphocytes is about the same in the exophthalmic goitre group as in the normal.

*Monocytes*

Table 4 shows the distribution of the monocytes in exophthalmic goitre. Obviously, almost all the patients had high monocyte counts. For example, only one patient had a count below Sabin's average normal of 5.7 per cent, whereas 26.5 per cent of the patients had counts over 15. This would indicate that relative monocytosis, to the extent of 2 to 3 times the normal value, is a definite and characteristic finding in the blood picture of exophthalmic goitre. The degree of monocytosis is expressed by the difference of  $8.4 \pm 0.81$  per cent between the normal and exophthalmic goitre values. This difference, being 10.4 times its standard deviation, is highly significant. The absolute number of monocytes in exophthalmic goitre is not increased as much as the percentage, the value being 916 cells as compared to the normal of 447 cells. The difference is large and significant.

*Eosinophiles and basophiles*

While an occasional case may show a high percentage of eosinophiles, such a finding is, however, not common. The average per cent of eosinophiles is lower than normal, but not significantly so. The absolute number is relatively lower than the percentage, namely, 214 cells against the normal of 345 cells. Although the difference seems large, it is probably not significant because the standard deviation of the normal eosinophile average is large.

There is no variation from the normal in the case of basophiles, either in per cent or in absolute number.

It is important to determine what relationship the white blood cell count and its differential picture have to the severity of the disease as measured by the basal metabolic rate, and to one another. There is no correlation whatever between the white blood cell count and the level of metabolism. The average count at different levels of metabolism varies a good deal, but in no consistent way.

There is a slight correlation between the percentage of polymorphonuclear cells and the metabolism, as indicated in Figure 2. The former tends to increase as the metabolism rises. For example, of the 12 cases with metabolic rates of 50 and over, 7 showed polymorphonuclear counts of 60 per cent or more, whereas only one of the 10 cases with metabolic rates under 40 showed this same percentage of polymorphonuclear cells. Mathematically this relationship, as expressed by the coefficient of correlation, is  $+0.324 \pm 0.115$ .

The lymphocytes, on the other hand, tend to vary inversely as the level of metabolism. This is also demonstrated graphically in Figure 2, which shows that the average lymphocyte percentage falls from 27.7 to 21.0 with the rise in level of metabolism. Expressing this fact in a different way, 4 of the 12 cases with metabolic rates of 50 and over had

15 per cent or less of lymphocytes whereas none of the remaining cases had this percentage of lymphocytes. The coefficient of correlation in this case is  $-0.39 \pm 0.109$ .

Figure 2 also shows the tendency of the monocytes to increase with the height of the basal metabolic rate. For example, 9 of the 18 cases with metabolic rates under 50 showed a monocyte count of 12 per cent or less, whereas only one of the remaining 12 cases showed this percentage of monocytes. The coefficient of correlation is  $+0.403 \pm 0.108$ —a value of probable significance.

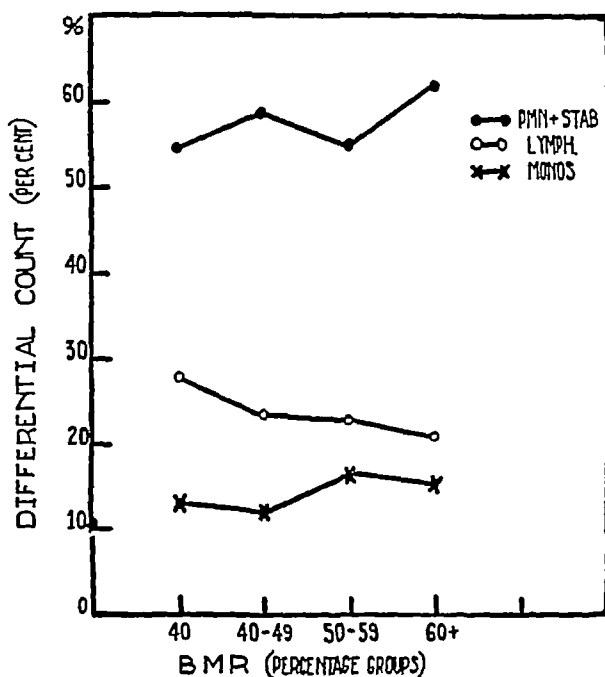


FIG. 2 THE AVERAGE PERCENTAGE OF NEUTROPHILES, LYMPHOCYTES AND MONOCYTES AT DIFFERENT LEVELS OF METABOLISM

In the above correlations the finding of a positive relationship between the neutrophile percentage and the height of the basal metabolic rate and a negative relationship between the lymphocyte percentage and the height of the metabolism was unexpected. Since hypopolynucleosis and relative lymphocytosis are common findings, one would expect a decrease in polymorphonuclears and an increase in lymphocytes as the metabolism increased. The explanation for this discrepancy is not clear.

It should be noted that the above discussion refers to the relative numbers of polymorphonuclears, lymphocytes and monocytes. When their absolute numbers are compared with the metabolic rates, all correlation is practically destroyed.

There is no significant correlation between the white blood cell count and its differential formula. On the other hand there is a highly signifi-

TABLE 6

*The blood picture and basal metabolic rate of 18 patients with exophthalmic goitre studied at different stages in the course of treatment*

Patient number	INITIAL LEVELS							LEVELS AFTER IODINE						
	Leuko-cytes	Poly morpho-nucleurs	Lympho-cytes	Monoc-ytes	Eosino-philes	Baso-philes	Basal metabolic rate	Leuko-cytes	Poly morpho-nucleurs	Lympho-cytes	Monoc-ytes	Eosino-philes	Baso-philes	Basal metabolic rate
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
I	5 000	55	25	14	5	1	+60	4 500	70	20	4	4	2	+28
II	9 200	67	15	18	0	0	+74	7 900	79	19	2	0	0	+25
III	5 000	64	18	12	5	1	+52	6 200	79	15	5	1	0	+36
IV	7 800	58	29	13	0	0	+61	6 000	67	25	4	2	2	+9
V	6 800	56	24	15	3	2	+46	7 400	76	18	3	3	0	+20
VI	6 850	40	38	18	4	0	+43	4 850	45	48	3	4	0	+20
VII	5 100	67	17	14	1	1	+56	5 200	73	17	5	5	0	+30
VIII	5 900	46	37	13	4	0	+38	5 800	80	16	3	1	0	+12
IX	5 450	53	31	12	4	0	+36	4 900	61	26	12	1	0	+14
X	7 150	64	14	19	3	0	+51	4 250	78	15	3	3	1	+15
XI	4 200	36	46	15	2	1	+52	6 800	81	14	3	1	1	+19
XII	6 250	53	28	16	2	1	+39	4 000	74	20	4	2	0	+16
XIII	5 000	52	24	17	5	1	+30	3 750	74	18	4	3	1	+15
XIV	6 250	56	30	12	2	0	+38	4 800	75	17	3	4	1	+12
XV	4 700	58	22	13	7	0	+30	3 300	76	18	3	2	1	+14
XVI	6 900	69	15	15	1	0	+40	3 800	82	10	3	3	2	- 3
XVII	5 700	56	26	14	3	1	+24	4 600	83	10	4	2	1	+24
XVIII	6 500	55	32	15	6	1	+42	5 400	72	20	4	3	1	+25

TABLE 6—continued

Patient number	LEVELS 1 DAY POSTOPERATIVE						LEVELS 5 TO 10 DAYS POSTOPERATIVE						
	Leuko- cytes	Poly- morpho- nuclears	Lympho- cytes	Mono- cytes	Eosino- philes	Baso- philes	Leuko- cytes	Poly- morpho- nuclears	Lympho- cytes	Mono- cytes	Eosino- philes	Baso- philes	Basal metabolic rate
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
I	5,900	68	18	12	1	1	4,200	83	14	1	2	0	+12
II	10,200	83	6	10	1	0	6,200	86	10	2	2	0	+10
III	12,300	77	12	10	0	0	6,500	82	15	3	0	0	+5
IV	9,000	84	8	8	0	0	6,800	76	17	4	3	0	+15
V	8,000	65	22	11	1	1	6,400	75	19	3	2	1	+6
VI	6,850	52	35	5	8	0	5,400	66	22	5	7	0	-17
VII	4,750	75	20	3	1	1	5,000	80	17	3	0	0	-15
VIII	9,100	71	17	8	2	2	5,800	75	19	2	4	0	+4
IX	12,000	81	12	6	1	0	4,400	70	25	2	3	0	+6
X	5,100	74	18	8	3	2	3,800	84	13	2	1	0	+14
XI	4,550	80	11	7	2	0	3,300	89	8	3	2	1	+2
XII	4,900	72	17	8	3	0	4,100	88	8	2	1	1	+8
XIII	5,200	75	16	4	4	1	3,050	83	10	3	3	1	-9
XIV	5,250	72	15	10	3	0	3,000	88	6	3	2	1	+7
XV	5,200	78	12	9	1	0	5,200	86	8	3	2	1	+2
XVI	8,300	89	8	3	0	0	5,500	79	17	0	3	1	-14
XVII	10,300	67	21	9	2	1	5,200	70	25	2	2	1	-5
XXVIII	7,200	84	12	2	1	1	7,200	89	7	1	1	2	+12

cant inverse relationship between the polymorphonuclear and lymphocyte percentages and between the lymphocyte and monocyte percentages

## II

## CHANGES RESULTING FROM IODINE AND OPERATION

Eighteen patients were studied under conditions of rest, iodination and following operation and their data are presented in Table 6. They include the levels of basal metabolism, white blood cell count, and differential formula of each patient. The postoperative period is divided into two in order to evaluate the effect of the postoperative febrile reaction and the final effect of operation.

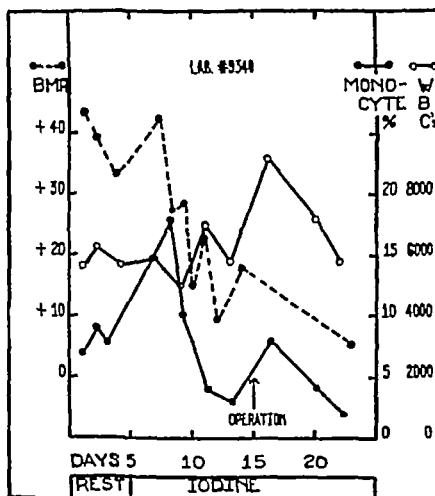


FIG 3 COURSE OF THE BASAL METABOLIC RATE, WHITE BLOOD CELL COUNT AND MONOCYTE PERCENTAGE OF A PATIENT WITH EXOPHTHALMIC GOITRE

Figure 3 represents graphically the typical course of the basal metabolic rate, white blood cell count, and monocyte percentage of a patient undergoing the usual treatment of rest, iodine and operation. It demonstrates clearly that changes in monocyte percentage follow closely the changes in metabolism. The rise in metabolism seen in this case in the first few days after the beginning of iodine medication is not uncommon. There was a concomitant rise in monocyte percentage. The total white blood cell count changed little except for the moderate increase after operation.

TABLE 7  
*Effect of rest, iodine and operation on the blood picture and basal metabolic rate of 18 patients with exophthalmic goitre \**

	AT REST		AFTER IODINE		AFTER OPERATION			
					1 DAY		10 DAYS	
	Per cent	Absolute numbers	Per cent	Absolute numbers	Per cent	Absolute numbers	Per cent	Absolute numbers
Polymorphonuclears	55.8 $\pm$ 1.84	3,447 $\pm$ 239	73.6 $\pm$ 2.06	3,828 $\pm$ 262	74.8 $\pm$ 1.98	5,613 $\pm$ 498	80.5 $\pm$ 1.63	4,049 $\pm$ 245
Lymphocytes	26.2 $\pm$ 2.01	1,534 $\pm$ 119	19.2 $\pm$ 1.91	976 $\pm$ 109	15.5 $\pm$ 1.53	1,090 $\pm$ 134	14.4 $\pm$ 1.40	695 $\pm$ 87
Monocytes	14.7 $\pm$ 0.50	909 $\pm$ 66	4.0 $\pm$ 0.49	204 $\pm$ 25	7.4 $\pm$ 0.68	563 $\pm$ 73	2.4 $\pm$ 0.26	123 $\pm$ 17
Eosinophiles	3.2 $\pm$ 0.46	163 $\pm$ 26	2.4 $\pm$ 0.31	120 $\pm$ 16	1.9 $\pm$ 0.44	124 $\pm$ 29	2.2 $\pm$ 0.37	112 $\pm$ 21
Basophiles	0.55 $\pm$ 0.14	31 $\pm$ 8.6	0.7 $\pm$ 0.17	34 $\pm$ 8.5	0.55 $\pm$ 0.16	39 $\pm$ 12	0.55 $\pm$ 0.14	28 $\pm$ 8.6
Total leukocytes		6,097 $\pm$ 286		5,192 $\pm$ 281		7,450 $\pm$ 585		5,058 $\pm$ 297
Basal metabolic rate	+45.1 $\pm$ 2.90		+18.4 $\pm$ 2.04				+2.4 $\pm$ 2.33	

\* Tabulation of the mean values and their standard deviations

Table 7 summarizes the data presented in Table 6 and will serve as the basis for our subsequent discussion. As in the original table, the averages are arranged in columns to demonstrate the separate effects of rest, iodine, and operation on the blood picture and basal metabolism.

The resting levels for this group of patients are essentially similar to those obtained for the large group of untreated patients listed in Table 1, and require no further discussion. They serve as controls for the data on the same patients obtained after iodination and operation. Rest, alone, as stated previously, had no appreciable effect on the blood picture even in those cases which showed considerable diminution in metabolic rate during the rest period.

Iodination caused the following changes from the resting levels:

1 In 14 of the 18 cases there was a definite decrease in the total of white blood cells, varying from 100 to 3,100 cells, the remaining 4 cases showed an increase. The average decrease as shown in Table 8 amounted to  $905 \pm 401$  cells or 15 per cent of the resting leukocyte count. This decrease is statistically significant.

2 There was a slight increase in absolute number of polymorphonuclear cells and a moderate increase in relative percentage. The latter increased in every case, whereas the former increased in 12 and decreased or was unchanged in 6. The average increase in absolute number of cells was  $381 \pm 355$  which is not significant, whereas the increase in percentage was  $17.8 \pm 2.76$  per cent which is highly significant (see Table 8).

3 Both the small and large lymphocytes decreased. There was a slight increase in lymphocytes in 2 cases only. The average change in the lymphocytic series was  $7.0 \pm 2.77$  per cent and  $558 \pm 161$  cells for the relative and absolute numbers respectively.

4 The monocytes showed the most marked decrease, both in percentage and in absolute number. All but one of the patients showed a drop to the normal level of 2 to 5 per cent. A reduction in absolute number of monocytes occurred in every case. The average change was  $10.7 \pm 0.70$  per cent and  $705 \pm 71$  cells, both values being highly significant. This represents the most characteristic change in the blood picture in exophthalmic goitre under the influence of iodine.

5 There were variable changes in the percentage and absolute number of eosinophiles and basophiles, but none of these were definitely significant.

Some of the changes in the blood picture under the influence of iodine present interesting relationships to the simultaneous change in basal metabolic rate. The monocytes, both absolute and relative numbers, showed a reduction somewhat proportional to that of the metabolic rate. The changes in total leukocytes, percentage of polymorphonuclears, and



TABLE 8  
*Summary of the changes produced by iodine and operation in the blood picture and basal metabolic rate of 18 patients with exophthalmic goitre*

	Change due to iodine		Change due to operative procedure		Final change due to thyroidectomy	
	Per cent	Absolute numbers	Per cent	Absolute numbers	Per cent	Absolute numbers
Polymorphonuclears	+17.8 ±2.76	+381 ±355	+1.2 ±2.86	+1,785 ±568	+6.9 ±2.63	+221 ±360
Lymphocytes	-7.0 ±2.77	-558 ±161	-3.7 ±2.45	+114 ±172	-4.8 ±2.37	-281 ±140
Monocytes	-10.7 ±0.70	-705 ±71	+3.4 ±0.84	+359 ±77	-1.6 ±0.56	-81 ±30
Eosinophiles	-0.8 ±0.56	-43 ±31	-0.5 ±0.54	+4 ±33	-0.2 ±0.48	-8 ±26
Basophiles	+0.15 ±0.22	+3 ±12	-0.15 ±0.23	+5 ±15	-0.15 ±0.22	-6 ±12
Total leukocytes		-905 ±401		+2,258 ±649		-142 ±408
Basal metabolic rate	-26.7 ±3.55				-16.0 ±3.10	

percentage and absolute number of lymphocytes showed no significant relationship to the changes in metabolism

The immediate effect of operation on the blood picture is the resultant of several complex factors. There was a definite rise in total leukocyte count ( $2,258 \pm 649$  cells) which was mainly due to the rise in the number of polymorphonuclear cells ( $1,785 \pm 568$ ) and monocytes ( $359 \pm 77$ ). All the other types of cells changed slightly. The increase in monocytes was out of proportion to that of the other cell types. They were the only ones which showed a significant change in the relative number of cells, i.e. an increase of  $3.4 \pm 0.84$  per cent.

The final state of the blood picture indicates that operation per se had relatively little effect upon it as compared to the effect of iodine. The polymorphonuclear cells increased slightly, the increase in percentage was statistically significant but the increase in absolute number was not. All the other cell elements decreased slightly, but only the lymphocytes and monocytes showed a significant change. Again the change in monocytes was proportionately larger than that of the other cells. The total leukocyte count decreased slightly ( $142 \pm 408$  cells) but not significantly. Operation changes the blood picture of iodimized patients relatively little, whereas it decreases their metabolism considerably ( $16.0 \pm 3.10$  per cent). However, one cannot say that operation, alone, would fail to bring about the entire change in the blood picture recorded above.

## DISCUSSION

The classification of white blood cells is much less arbitrary in the supravital technique than in the fixed smear. Differences in the techniques have been pointed out by Sabin, et al in their quantitative studies of the daily rhythm of the white blood cells (15). Sources of error, which they thought were greatly reduced in the newer method, were the elimination of the "smudges" of fixed smear and the misclassification of the small monocytes, large lymphocytes and monolobed granular cells. It is not unusual to record an increase of 5 to 10 per cent in the percentage of monocytes by the supravital technique over that obtained by the fixed smear in cases of exophthalmic goitre.

Our experience with the various methods of blood study indicates that this misclassification of the monocytes in fixed smears may be the explanation of the apparent discrepancies found in the literature on the blood picture in exophthalmic goitre.

The effect of iodine on the blood picture in exophthalmic goitre is definite and almost constant. This stands in marked contrast to the findings reported by Jackson (12). His data, in addition to the fact that they were obtained by the fixed smear method, are subject to other sources of error. There is no indication that the monocytes were classified as a separate group. Moreover, conclusions are drawn from average

values without due consideration for the variability of the results or the standard deviation. It seems to us that Jackson obtained the post-iodine counts before the patients were fully iodinated because the reduction of 12 points (plus 46 to plus 34) in the basal metabolic rate of his group of cases is much smaller than the 30 or more point drop reported from other clinics, including our own. Consequently he attributes certain changes in the final blood picture to operation, which are probably due to iodine.

The elevated monocyte count in exophthalmic goitre and its depression by iodine in the course of the remission produced by this drug may be of fundamental importance in our understanding of the pathogenesis of the disease. If Aschoff's view that the monocyte of the circulating blood is part of the "reticulo-endothelial metabolic apparatus" (16) is correct, then our results suggest that the reticulo-endothelial system is activated in hyperthyroidism and that the remission produced by iodine is linked with a depression of this system. At the present time we are engaged in further experiments in an attempt to establish the validity of these suggestions.

### CONCLUSIONS

- 1 The supravital technique offers a valuable aid in the study of the blood picture in exophthalmic goitre.

- 2 The discrepancies in the literature as to the blood picture in exophthalmic goitre are probably due to misclassification of the monocytes, a source of error which is reduced to a minimum by the use of the supravital technique.

- 3 The most marked and characteristic finding in the blood of patients with exophthalmic goitre is a relative and absolute monocytosis. Leukopenia and hypopolynucleosis are the rule. The percentage of lymphocytes is increased above normal in most cases, but relative lymphocytosis (of 30 per cent and over) occurred in only about a quarter of the cases. The absolute number of lymphocytes is normal.

- 4 The basal metabolic rate shows a direct correlation of probable significance with the percentage of monocytes and of polymorphonuclears respectively, and an inverse correlation of the same order with the percentage of lymphocytes. There is no relationship between the level of metabolism and the total leukocyte count.

- 5 The most characteristic effect on the blood picture of exophthalmic goitre from the administration of iodine is a reduction both in percentage and absolute number of monocytes. In addition, there is a moderate decrease in percentage and absolute number of lymphocytes, and a significant increase in the percentage but not in the absolute number of neutrophils. The total number of leukocytes decreases in most cases.

- 6 The reduction in the number and percentage of monocytes tends to be proportional to the reduction in basal metabolic rate. The changes

in the other cell elements and in the total leukocyte count do not show any such relationship to the basal metabolic rate

7 The immediate effect of operation is to increase the total number of leukocytes, due chiefly to the increase in the number of polymorphonuclear cells and monocytes. Only the percentage of monocytes is significantly changed

8 The final changes in the blood picture following operation are qualitatively the same as those produced by iodine but are much smaller. The total white blood cell count is reduced slightly but not significantly. The reduction in metabolism is proportionately greater than the change in the blood picture

9 The possibility of depression of an activated reticulo-endothelial system in exophthalmic goitre by iodine is suggested by the data presented and is discussed briefly in the light of Aschoff's view of the origin of the monocyte

We are indebted to Dr James H Means for supervision of this work and to Dr Carl F Doering, of the Department of Vital Statistics, School of Public Health, Harvard University, for guidance in the statistical treatment of the data

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# EXPERIMENTAL OBSERVATIONS ON THE EFFECT OF VARIOUS DIURETICS WHEN INJECTED DIRECTLY INTO ONE RENAL ARTERY OF THE DOG<sup>1</sup>

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In the study of the action of diuretics the first question to determine is whether the drug acts directly on the kidney, or indirectly through changes in the blood or in the circulation. Since the publication of the theories of urine secretion advanced by Bowman, Heidenhain and Ludwig, an extensive literature has sprung up in an effort to add light to this problem. Experimental results have, however, been conflicting.

Von Schroeder (1) in 1888 first examined the action of caffeine, theobromine and theophylline on the kidney of the rabbit, cat and dog. He felt that diuresis was due to a specific stimulation of the kidney cell, and was independent of circulatory effects. Barcroft and Straub (2) also favored the secretion theory since they found that caffeine and urea increased the metabolism of the kidney of the rabbit and cat during the period of diuresis. However, Hayman and Schmidt (3), using caffeine, were unable to confirm these results. Richards and Plant (4), working with the kidney kept alive by a special form of perfusion, found that caffeine produced a diuresis when the kidney blood flow and volume remained constant. Cushny and Lambie (5) studied the effect of the intravenous injection of caffeine, urea and strophanthin on the change in per minute blood flow through the kidney of the rabbit. They found that caffeine and urea produced a short stage of acceleration of blood flow, soon followed by a return to normal rate, while the diuresis persisted. Under the same conditions small doses of strophanthin produced no appreciable change in blood flow and had no diuretic effect, larger doses decreased the blood flow through the kidney and diminished the urine output. They concluded that the diuretic action of caffeine and urea was independent of any change in blood supply. They believed that caffeine caused diuresis by reducing the resistance to filtration through the glomerular capsule, by a specific action on its cells, and that urea acted by increasing the osmotic resistance to the re-absorption of filtrate in the tubules.

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Loewi, Fletcher and Henderson (6) opposed the secretion theory of Von Schroeder and held that the primary action of caffeine was one of renal vasodilatation, which tended to increase filtration through the capillaries Phillips and Bradford (7), Gottlieb and Magnus (8) and Starling (9), while studying changes in kidney volume by means of the oncometer, found that caffeine diuresis was accompanied by a persistent and marked expansion. However, as Cushny has suggested, the kidney volume depends not only on the calibre of the blood vessels, but also on the contents of the renal tubules and the amount of lymph in the interstices. Variations in any or all of these factors would tend to influence the oncometer readings.

Hayman and Starr (10), in estimating the number of functioning glomeruli in the rabbit's kidney by intra-vital staining methods, found that during the action of caffeine all glomeruli were open. This work has been substantiated by that of Richards and Schmidt (11), who showed, while observing directly the circulation through the glomeruli in the frog's kidney, that the number of active glomeruli and the degree of activity were increased by caffeine. This would tend to increase the capillary surface and hence the extent of the filtering bed.

Spiro and Vogt (12) found that, in addition to a diuretic action, caffeine produced a marked increase in lymph flow through the thoracic duct. They assumed that the drug had an action on tissue exchange which was independent of the renal action. Spiro (13) studied the effect of theocine on rabbits and found that a single large dose produced first a polyuric and later an oliguric stage. During the polyuric stage there was a decrease in the sodium chloride per cent in both the urine and blood, and the water content of the blood was lessened. During the oliguric stage the concentration of the blood gradually returned to normal. In nephrectomized animals the drug produced an absolute decrease in water and chloride content of the blood. He concluded that the purine bases, as well as having an action on the kidney, also had a direct effect on tissue exchange, by means of which the tissues took up water and sodium chloride from the blood.

Ellinger, Heymann and Klein (14) recently advanced a chemical theory in which they held that the purine derivatives decreased the affinity of the plasma proteins for water and thus favored filtration through the glomeruli. This view, however, has received little support.

Many investigators believe that the diuretic action of mercury is dependent on changes in the blood or tissues, some support the theory of a direct renal action, while others hold that both factors exist. The work of Govaerts (15) strongly favors the theory of a direct action on the kidney. In one group of experiments the kidney from a dog at the height of novasurol diuresis was transplanted to the neck of a normal dog, anastomosing the renal artery to the carotid artery, and the renal vein to

the jugular vein. At the completion of anastomosis an abundant urine, seven to one hundred times greater than that from the normal kidney *in situ*, was obtained. This urine had all the characteristics of novasurol urine. In another group of experiments a kidney from a normal dog was transplanted to the neck of an animal at the height of novasurol diuresis, the urine output from the neck kidney was ten to one hundred times less than that from the novasurolized kidney in place. The urine from the transplanted kidney was highly colored and lacked the characteristics of novasurol urine. From these experiments Govaerts concluded that the action of novasurol was primarily on the kidney. Schmidt (16) perfused the frog's kidney with small doses of novasurol and found that the drug produced a distinct diuresis. Gremels (17), using the heart lung preparation of Starling and Verney in irrigating the dog's kidney, found that if he added to the blood of such a preparation 0.01 to 0.04 gram of novasurol or 0.025 gram of salyrgan a marked diuresis was produced, and this without change in the volume blood flow through the kidney. Melville and Stehle (18), believing that novasurol was only indirectly active through its decomposition products, made use of mercuric chloride. They used dogs, injecting the drug into one renal artery and measuring the urine output from both kidneys. The resulting diuresis began simultaneously from both sides, and in some instances the output from the injected kidney was diminished. They concluded that mercury produced its diuretic effect through an extra-renal action. Several German investigators, including Nonnenbruch (19), Tezner (20), Saxl and Heilig (21) and Bohn (22), believe novasurol to act largely on the tissues and plasma colloids.

Since the introduction of digitalis into medical therapeutics there has been considerable controversy regarding its diuretic action. Some believe that the drug has a direct effect on the kidney and its vessels, while others hold that diuresis is the result of an improved general circulation. Reinike (23) investigated the action of digitalis on the rabbit's kidney by administering the drug to a group of animals over a long period of time. He found the kidneys of these animals to be larger than those of a control series and suggested that under the influence of the drug the kidneys had undergone excessive activity. Phillips and Bradford (7) found that digitalin produced a decided increase in urinary flow when administered to the dog, this was accompanied by a peripheral vascular constriction as indicated by the oncometer. Strophanthin had little effect either on the vessels of the kidney or the urinary output. Kasztan (24) perfused the kidney of the dog, cat and rabbit with Ringer's solution. When small doses of strophanthin were added to the circulation, arterial dilatation took place, larger doses caused constriction. He concluded that strophanthin had a peripheral effect on the vessels of the kidney. Fahrenkamp (25) obtained identical results using digitoxin in place of strophanthin. Gremels,



whose work has been referred to, concluded that strophanthin and digitoxin had an action on the renal parenchyma quite separate from that on the peripheral vessels

The following experiments were performed on dogs in the laboratory of the Department of Medicine of Harvard Medical School, at the suggestion and under the supervision of Dr Henry A Christian, Hersey Professor of the Theory and Practice of Physic in Harvard University. The object of the work was to study the effect of the injection of diuretics in varying dosage into one renal artery. It was felt that, if the diuretic had a direct action on the renal parenchyma, a dose sufficiently small should produce a diuresis from the injected kidney and leave the opposite kidney unaffected (since it could not reach the other organ in the same concentration), a slightly larger dose should produce a bilateral effect, manifested earlier and being more marked on the injected side, while a still larger dose might shut down the injected kidney and from the other induce a diuresis.

#### EXPERIMENTAL METHODS

The dogs used in this work were healthy young animals. They were allowed water but deprived of food for a period of eighteen hours prior to the beginning of the experiment.

Anesthesia was induced by the intraperitoneal injection of dial "Ciba"<sup>2</sup> with urethane, in doses of 0.65 cc per kgm of body weight. This dosage was sufficient to cause complete anesthesia in 20 to 30 minutes, the effect persisting throughout the duration of the experiment. Only rarely was it necessary to use additional small doses of dial. The animal was placed on a warm padded operating table. The abdomen was opened by a low midline incision and the bladder delivered to the surface. The ureters, at the point of entry into the bladder, were isolated and dissected free for a distance of approximately 1 cm. A small incision was made into each ureter at an avascular point and flexible ureteral catheters of equal diameter inserted. These were held in place and leakage prevented by a ligature of number 0 chromic catgut. The bladder was emptied and returned. The peritoneum, the muscle wall and the skin were approximated in three layers, by a continuous suture of black silk, allowing the distal ends of the catheters to escape from the lower part of the incision. The above procedure usually required twenty to thirty minutes.

Urine was collected from each catheter into graduated cylinders and measured at half-hourly intervals. Hydremia was maintained by the intravenous injection of 100 to 200 cc of 0.9 per cent NaCl solution every thirty minutes. The solution was warmed to body temperature and injected slowly from a 100 cc syringe at the rate of 50 cc per minute into one of the leg veins.

Because of the relative ease in exposure, the left renal artery was chosen for injection of the diuretic. With the animal on the right side, an infracostal incision was made through the skin and muscles of the left flank. The peritoneum was gently drawn aside and the renal artery isolated, care being taken

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<sup>2</sup> We wish to express our thanks and appreciation to the Ciba Company, Greenwich and Morton Streets, New York City, for supplying us with the anesthetic used in this work.

to avoid undue trauma to the nerves running along the artery, to the renal vein and the ureter. The artery was supported by a loosely held ligature of number 0 catgut. The diuretic was injected slowly through a small needle, on the withdrawal of which bleeding was controlled by light pressure with a gauze pack. During injection the blood supply to the kidney was disturbed as little as possible, and at no time was shut off for more than a fraction of a minute. The muscles and skin were approximated with a continuous suture of black silk.

The duration of each experiment was  $5\frac{1}{2}$  to 6 hours, a control period of  $2\frac{1}{2}$  to 3 hours preceding the injection of the diuretic. At the termination of the experiment the animal was sacrificed, and the catheters and ureters examined in order to rule out any factors which might interfere with urine flow.

Examination of the urine included specific gravity determinations, and estimation of albumin by the Heller test.

### RESULTS

This study included eighty-two experiments from which typical examples have been selected as illustrative of the action of each diuretic used.

Since the experiments were acute in nature, it was necessary to study the relation of the anesthetic to urine output. In Figure 1 the results of the intravenous injection of 200 cc. of 0.9 per cent NaCl solution every half-hour upon the rate of urine excretion in (a) the normal unanesthetized dog, and (b) the dog anesthetized by the intraperitoneal injection of dial are well shown. In the normal dog an enormous diuresis was produced, whereas in the anesthetized animal the output at hourly intervals never exceeded 40 cc. This depicts the inhibitory rôle played by the anesthetic throughout these experiments.

Figure 2 shows the results obtained by the injection directly into the left renal artery, of small doses of the organic mercury compounds (salyrgan and novasurol). With the dosage used, the injected kidney showed a very definite and prompt diuresis, while the output of the opposite kidney did not increase. In the experiment with novasurol, shown in the upper set of curves, there was difficulty in exposing the main artery, only one of the two branches could be injected. This undoubtedly explains the relatively slight diuretic effect. The response to slightly larger doses is illustrated in Figure 3. It is noted that both kidneys showed a well marked diuresis, which was earlier in onset and more profuse from the injected side. Figures 4 and 5 present the results of still larger doses, with these dosages the output of the injected kidney was diminished, while that of the opposite side was increased enormously. The observations with theocine-sodium acetate are shown in Figure 6, the response to this drug resembled in kind but was less marked than that with the mercury compounds. In the experiments in which small doses of salyrgan, novasurol and theocine-sodium acetate were used, the urine from the injected kidney was pale in color, of a low specific gravity and showed albumin, while that from the opposite kidney remained well colored, of a

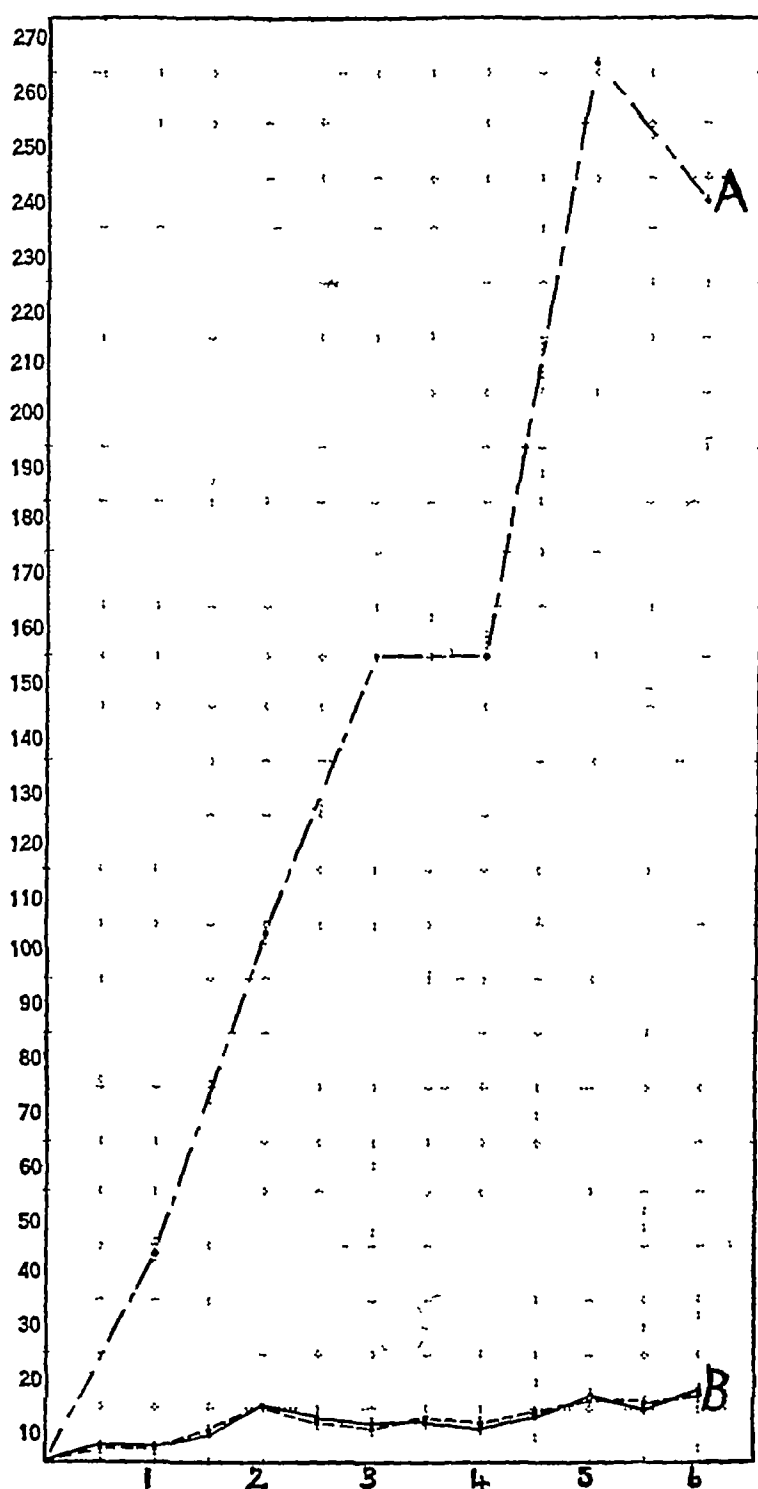


FIG 1 A Unanesthetized dog, urine output in cc per hour B Anesthetized dog, solid line shows urine output in cc per half hour from left kidney, broken line shows urine output in cc per half hour from right kidney

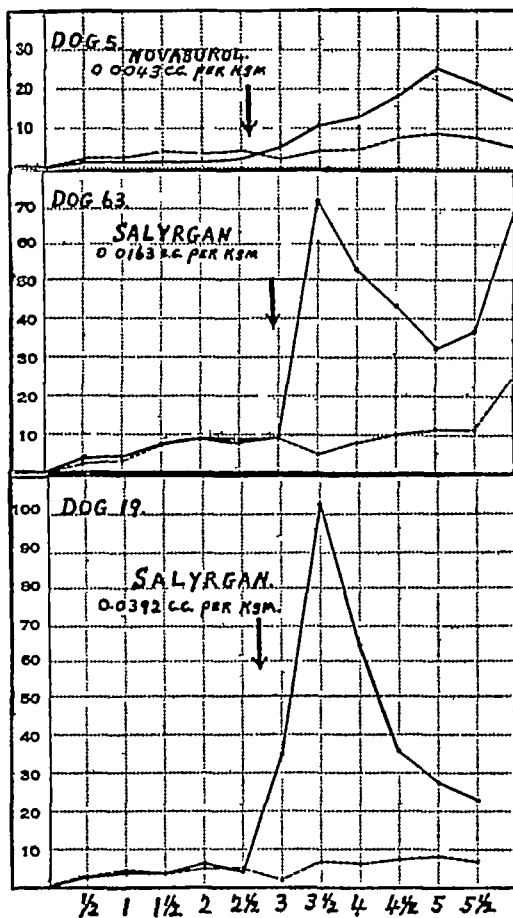


FIG 2 Solid line shows urine output in cc. per half hour from left kidney (injected kidney) broken line shows urine output in cc. per half hour from right kidney Arrow indicates injection of diuretic.

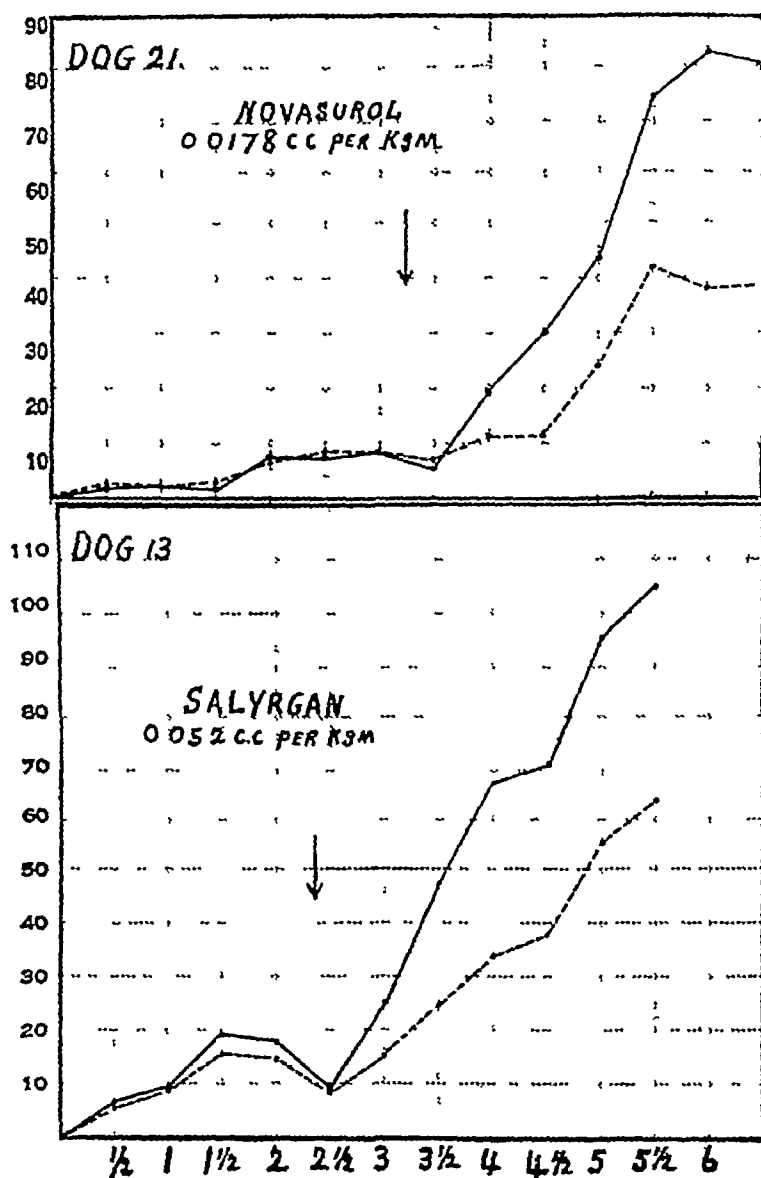


FIG 3 Solid line shows urine output in cc per half hour from left kidney (injected kidney), broken line shows urine output in cc per half hour from right kidney. Arrow indicates injection of diuretic.

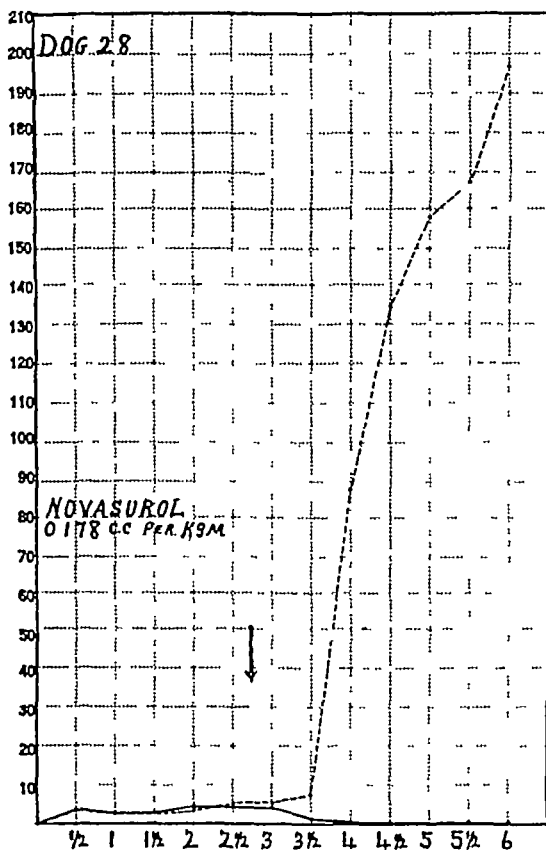


FIG 4 Solid line shows urine output in cc. per half hour from left kidney (injected kidney), broken line shows urine output in cc. per half hour from right kidney. Arrow indicates injection of diuretic.

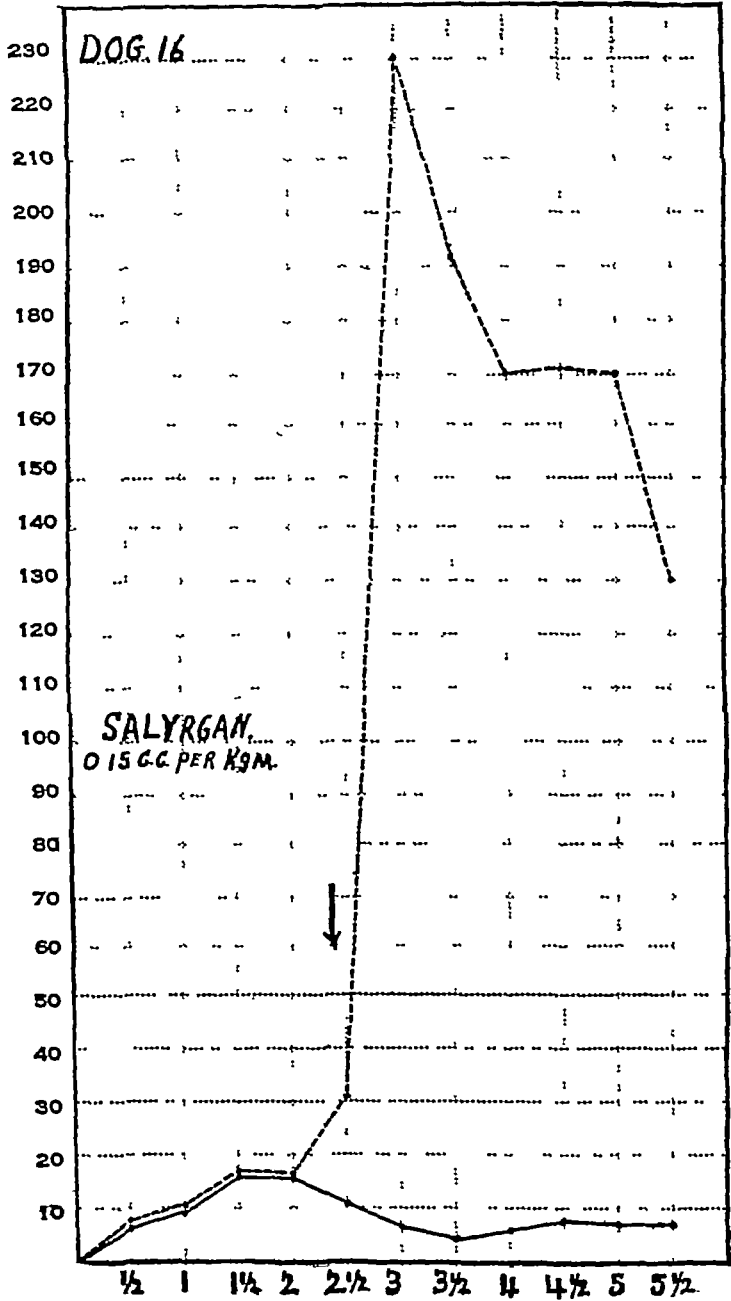


FIG 5 Solid line shows urine output in cc per half hour from left kidney (injected kidney), broken line shows urine output in cc per half hour from right kidney Arrow indicates injection of diuretic

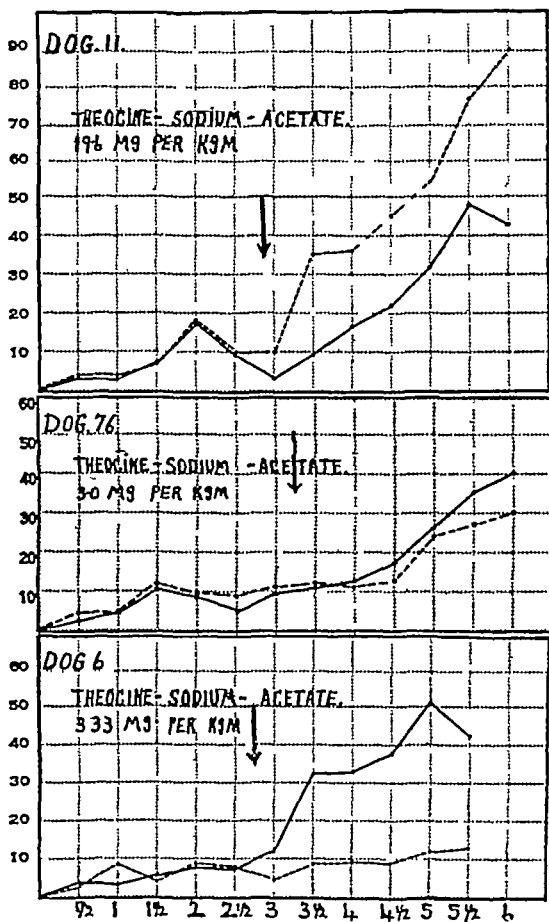


FIG 6 Solid line shows urine output in cc. per half hour from left kidney (injected kidney), broken line shows urine output in cc. per half hour from right kidney. Arrow indicates injection of diuretic.



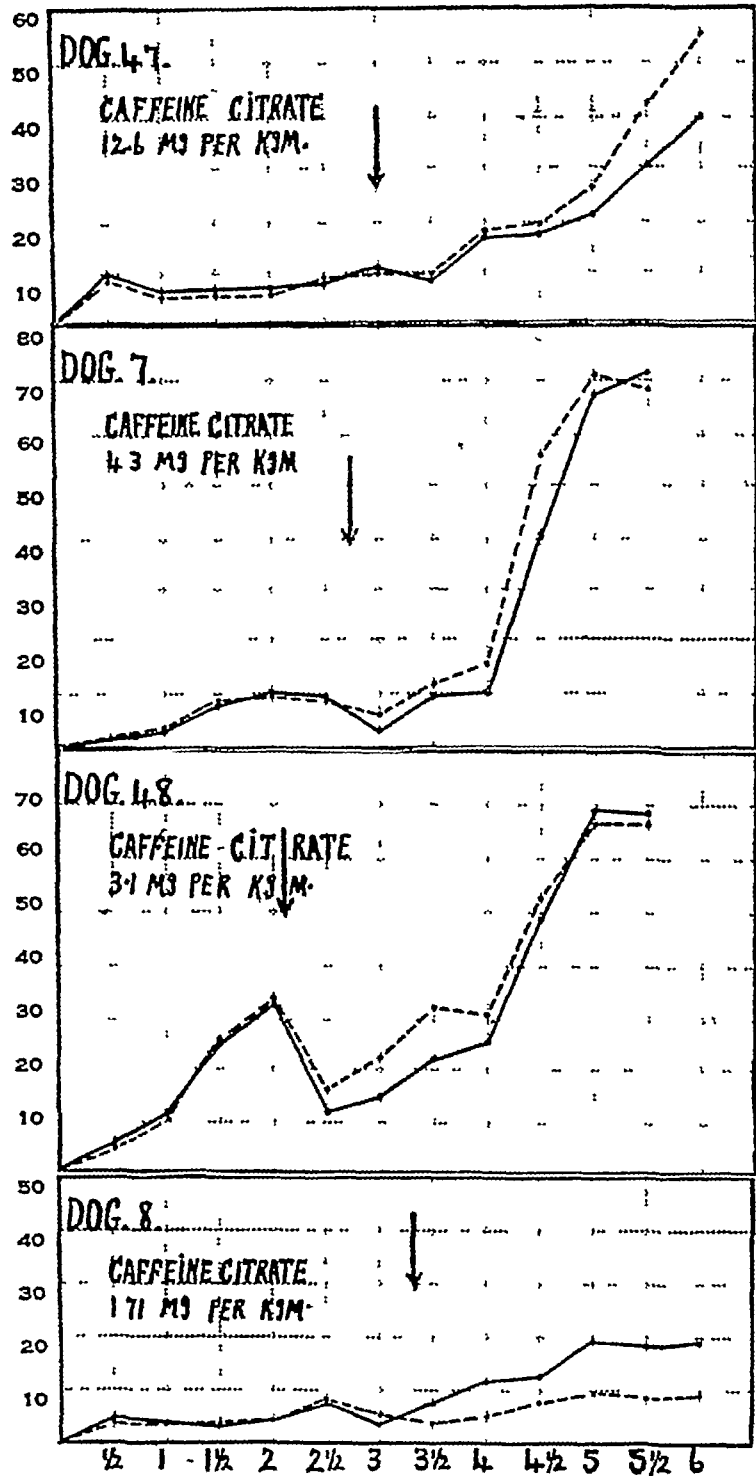


FIG 7 Solid line shows urine output in cc per half hour from left kidney (injected kidney), broken line shows urine output in cc per half hour from right kidney. Arrow indicates injection of diuretic.

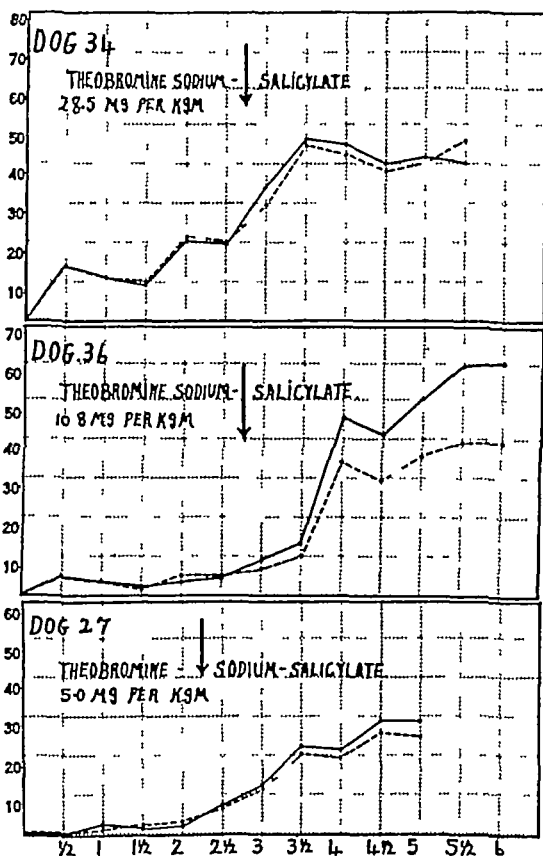


FIG 8 Solid line shows urine output in cc. per half hour from left kidney (injected kidney) broken line shows urine output in cc. per half hour from right kidney Arrow indicates injection of diuretic.

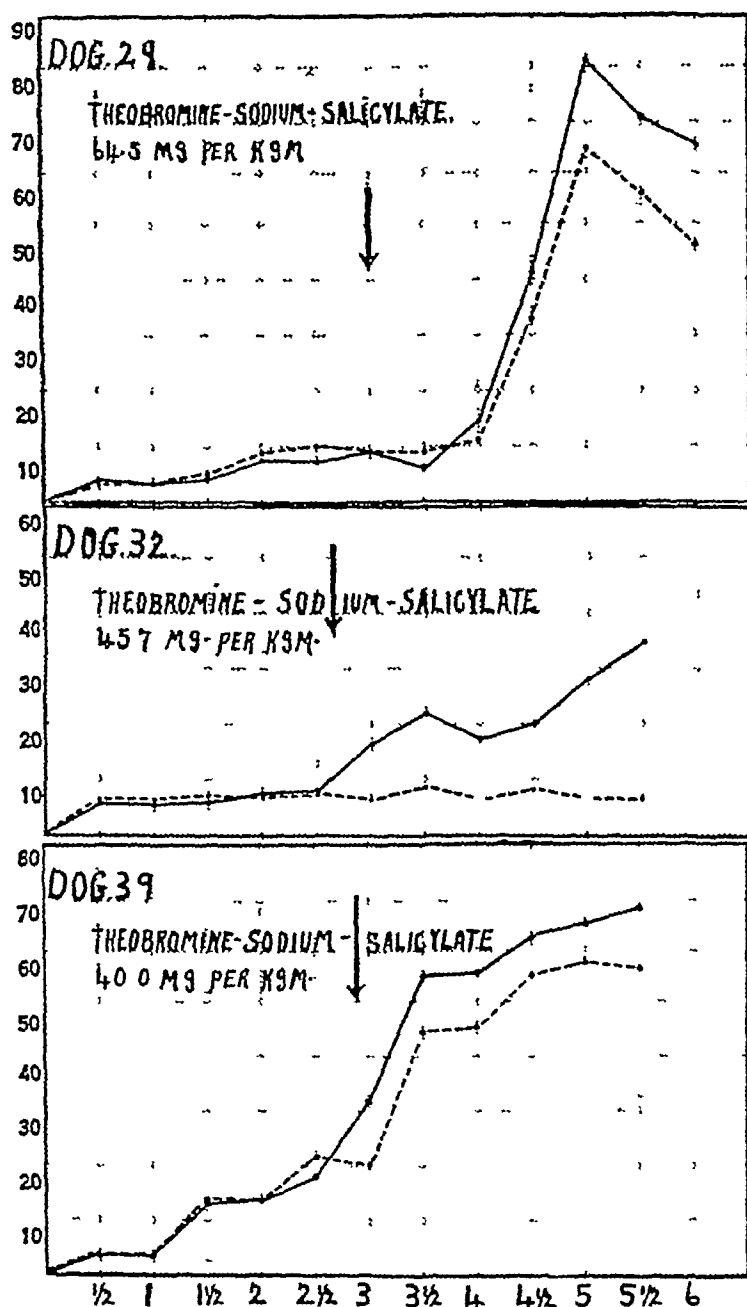


FIG 9 Solid line shows urine output in cc per half hour from left kidney (injected kidney), broken line shows urine output in cc per half hour from right kidney. Arrow indicates injection of diuretic.

considerably higher specific gravity, and showed no albumin or only the slightest possible trace. With larger doses of the same diuretics the urines from both kidneys were identical in color and specific gravity, but that from the injected kidney showed relatively more albumin.

Figures 7 to 13 indicate the results observed with the injection of varying doses of caffeine-citrate, theobromine-sodium salicylate,<sup>3</sup> theophylline-ethylene-diamine,<sup>4</sup> theophylline-calcium salicylate and urea

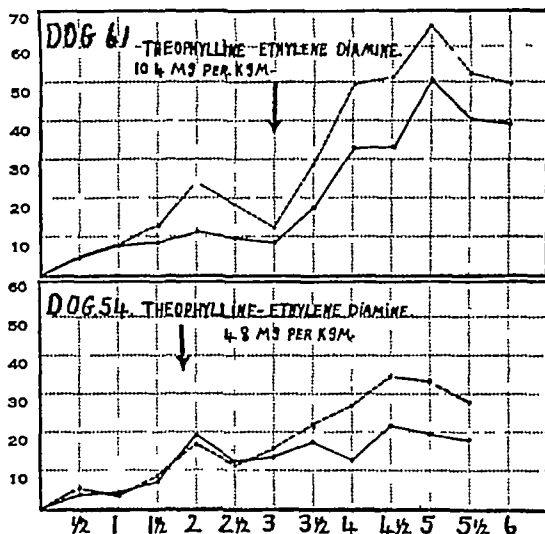


FIG 10 Solid line shows urine output in cc per half hour from left kidney (injected kidney) broken line shows urine output in cc. per half hour from right kidney. Arrow indicates injection of diuretic.

It is noted that this group of diuretics produced a bilateral response, the time interval between injection and onset of diuresis showing little difference as regards the two kidneys. In some of these experiments diuresis commenced a few minutes after injection of the diuretic, in others a considerable latent period was noted. The effect of digitan is shown in

<sup>3</sup> We wish to express our thanks and appreciation to Bilhuber Knoll Corporation, 154 Ogden Avenue, Jersey City, New Jersey, for supplying us with theobromine sodium salicylate (diuretin) and theophylline-calcium salicylate (phyllcin) used in these experiments.

<sup>4</sup> We wish to express our thanks and appreciation to A. W. Kretschmar, Inc., 396-398 Broadway, New York City, for furnishing us with theophylline-ethylene-diamine (theophylline) used in this work.

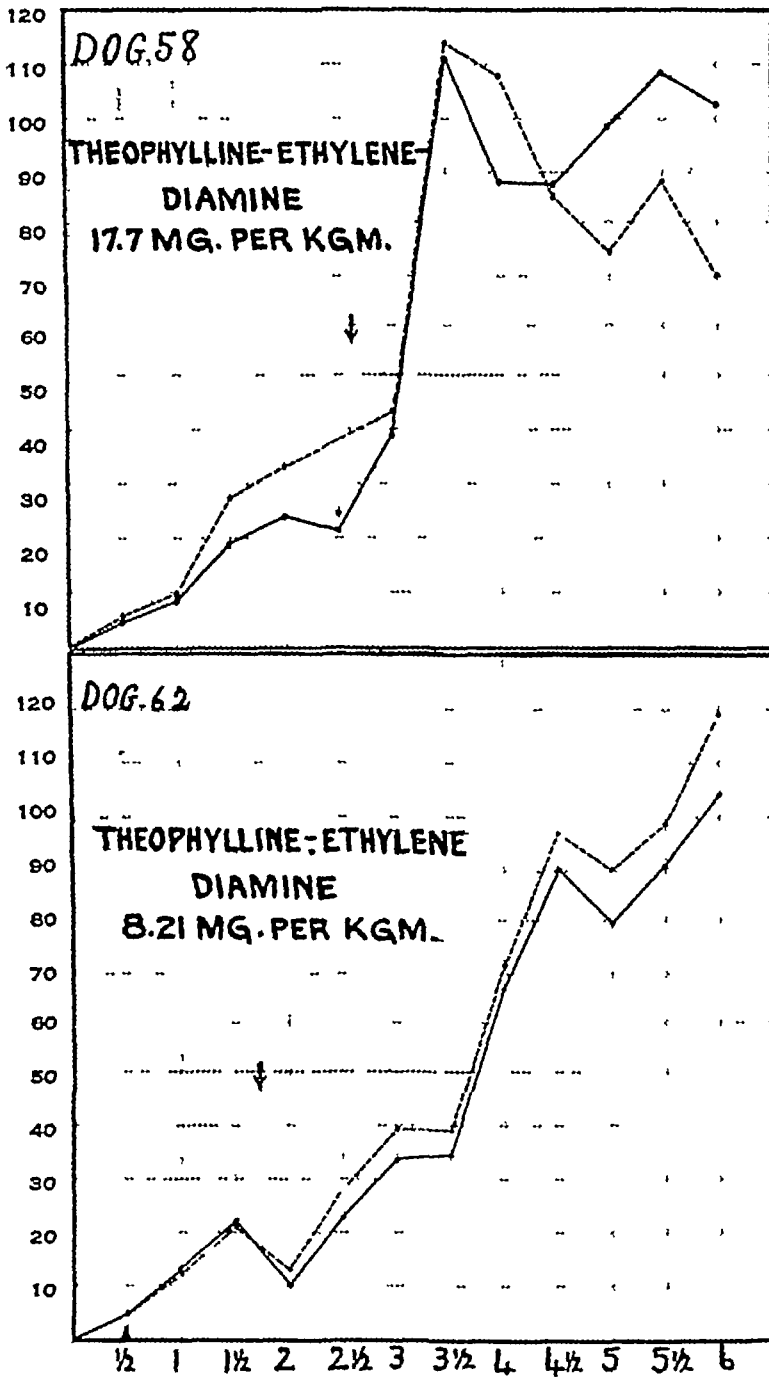


FIG 11 Solid line shows urine output in cc. per half hour from left kidney (injected kidney), broken line shows urine output in cc per half hour from right kidney Arrow indicates injection of diuretic

Figure 14 A bilateral diuresis occurred, but the output of the injected kidney was relatively less, particularly when larger doses of the drug were used. In this group of experiments the urines from the two kidneys were practically identical during the period of diuresis. They were alike in color, of an equal specific gravity, and showed similar amounts of albumin (slightest possible trace to very slight trace)

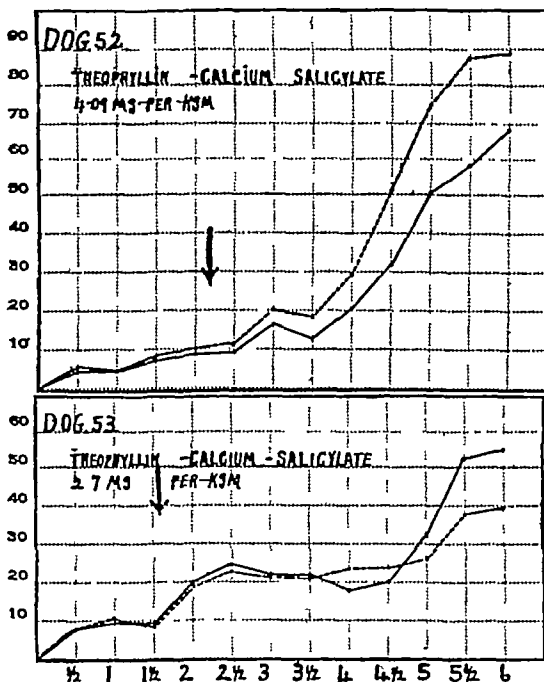


FIG 12 Solid line shows urine output in cc. per half hour from left kidney (injected kidney) broken line shows urine output in cc. per half hour from right kidney. Arrow indicates injection of diuretic.

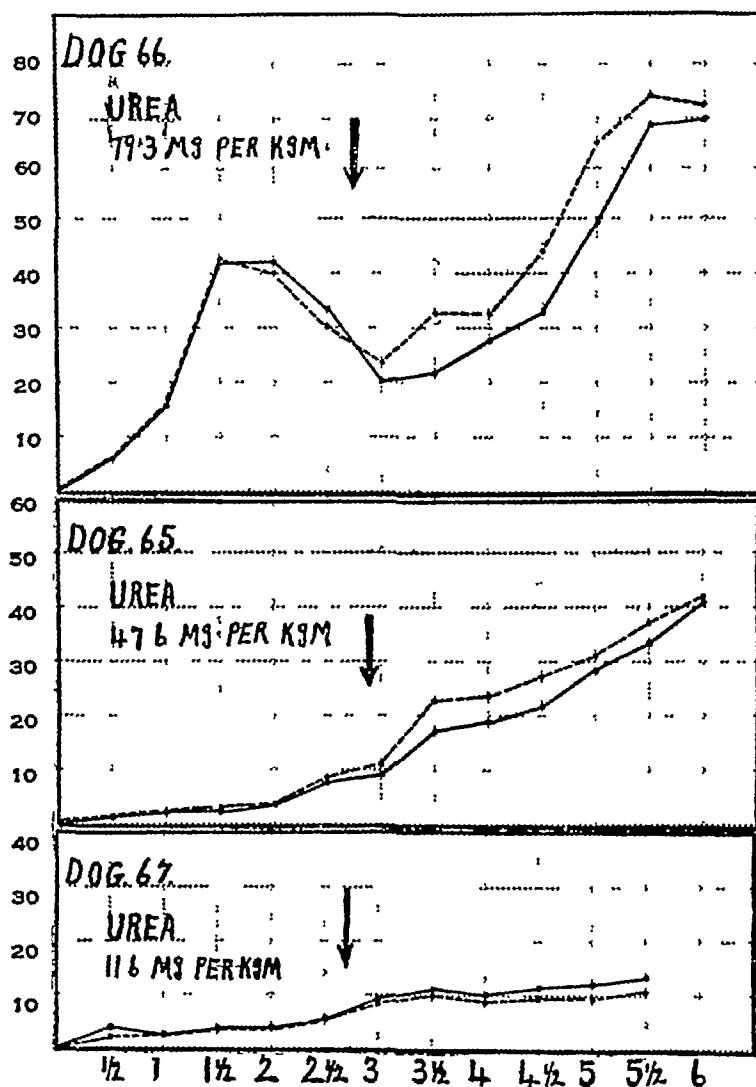


FIG 13 Solid line shows urine output in cc per half hour from left kidney (injected kidney), broken line shows urine output in cc per half hour from right kidney. Arrow indicates injection of diuretic.

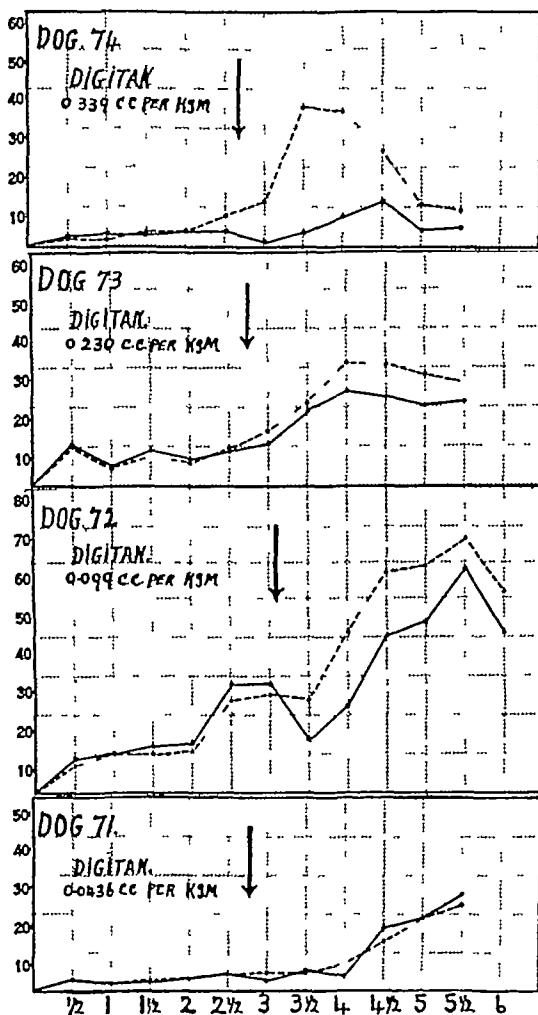


FIG 14 Solid line shows urine output in cc. per half hour from left kidney (injected kidney) broken line shows urine output in cc. per half hour from right kidney Arrow indicates injection of diuretic.



## DISCUSSION

Von Schroeder (1), who first investigated the diuretic action of the purine group, came to the conclusion that the dog's kidney was refractory to caffeine, since he failed to obtain a diuretic response. Wallace and Pellini (26), working with dogs in a state of water balance, and nitrogen and salt equilibrium, found that the caffeine group actually diminished the daily urine output. However, a review of their experiments shows that the urine was collected by catheterization at twenty-four hourly intervals. It seems quite possible that although the daily urine output was diminished, there may have been a period of diuresis with subsequent oliguria, in which case the total output for the day might not necessarily be increased. Loeb (27) also was unable to obtain a definite diuresis by administering caffeine to the normal dog, but noted that when hydremia and diuresis were induced by a constant infusion of saline solution, the secretion of urine was increased to a marked degree.

In our experiments hydremia was maintained by the intravenous injection of 100 to 200 cc of 0.9 per cent NaCl solution every half-hour. That a good deal of this fluid was stored in the tissues there is little doubt, for as Bogert, Underhill and Mendel (28) have shown, complete restoration of original blood volume in the rabbit and dog, takes place in thirty minutes after the intravenous injection of a quantity of saline solution equal to the calculated blood volume of the animal. They estimated also that the capacity of the tissues of the rabbit to absorb fluid was four times the normal blood volume of the animal.

In the normal dog this amount of saline solution should induce an enormous diuresis, probably as Cushny has suggested by dilution of the plasma proteins, which results in a more profuse filtration through the glomeruli. However, in our experiments which were conducted not on the normal dog, but on the dog subjected to prolonged anesthesia, a dilution diuresis appears to have played an insignificant part. This is probably attributable to the inhibitory action of the anesthetic, an action which has been observed by many workers, and one which seems impossible to overcome in acute animal experimentation. Fee (29) has shown that water diuresis in decerebrate dogs is markedly diminished by the administration of various anesthetics, the inhibition lasting for the same length of time as the narcotic effect. Ogden (30) made similar observations, using as an anesthetic full doses of amytal by the intraperitoneal route. This inhibitory action is well shown in Figure 1, where the infusion of 200 cc of 0.9 per cent NaCl solution at half-hour periods in the normal dog gave rise to a marked diuresis, whereas the dog under dial anesthesia showed a constant output with no tendency to diuresis. In a few other experiments, particularly with the purine group of diuretics, where for some reason or other the diuretic failed to act, the same more or less constant urine output continued throughout the duration of the

experiment, there being little tendency to diuresis from the injected saline. These observations justify the assumption that the diuretic effects obtained in our experiments were precipitated by the injection of the diuretic, and were not the result of a spontaneous diuresis from the injected saline solution, although the latter may have augmented the diuretic effect of the former.

From our experimental results we feel justified in dividing the diuretics used in this work into two main groups, (1) those that appear to exert their primary effect by a direct action on the renal parenchyma, and (2) those that appear to have a different and possibly an extra renal action.

Included in the first group are the organic mercury compounds (novasurol and salyrgan), also one of the xanthine derivatives (theocine-sodium acetate). When small doses of novasurol and salyrgan are injected directly into one kidney an immediate and well marked diuresis ensues from the injected side, the urine immediately changes from one of high color to one of pale color, shows albumin and has a lower specific gravity, whereas the urine from the opposite kidney retains its color, shows no albumin and no change in specific gravity. Slightly larger doses induce a bilateral diuresis, more marked and earlier in onset from the injected side, the urines from both kidneys being pale and of an equally low specific gravity, that from the injected kidney showing more albumin. Still larger doses shut down the injected kidney and produce a marked diuresis from the opposite side, the urine from the injected kidney showing a considerable amount of albumin (slight trace to trace). The probable explanation of these results is that salyrgan and novasurol induce diuresis by a direct action on the renal parenchyma, possibly involving the tubules with resulting decreased absorption. The action of theocine-sodium acetate is similar to that of the mercurial drugs, although the diuretic effect is less marked.

The second group includes caffeine-citrate, theobromine-sodium salicylate, theophylline-ethylene-diamine, theophylline-calcium salicylate, urea and digitan. The injection of any of these diuretics in varying doses results in a bilateral diuresis, beginning in some instances a few minutes after injection, in others after a latent period has intervened. Both kidneys respond to about the same degree, and the urines from both sides are identical. It is noted that in the experiments in which theobromine sodium salicylate was used, the diuresis from the injected kidney was always a little greater than that from the opposite side, and in one experiment, Dog 32, Figure 9, the injected kidney showed a moderate diuresis, while the opposite kidney failed to respond, the urine from the injected side was pale in color and of a much lower specific gravity. With theophylline-ethylene-diamine, the output from the injected kidney was usually slightly less than that from the opposite kidney. Urea and digitan are less effective than the other diuretics included in this group. With digitan, particularly in larger doses, the diuresis from the in,

side was somewhat less in degree. This may be due to the peripheral vasoconstrictor effect of the digitalis series on the renal vessels. It seems logical to conclude from the study of this group, that the mechanism of diuresis differs from that induced by the mercurial compounds (novasurol and salyrgan) and by one of the xanthine group (theocine-sodium-acetate), and that possibly an extra-renal site of action may be an important factor in the response to these drugs. Further investigation will be needed to explain satisfactorily the definite mechanism of diuresis from these two apparently differently acting groups of diuretics.

### CONCLUSIONS

From observations on acute experiments with the anesthetized dog, rendered hydremic by the intravenous injection of saline solution, we draw the following conclusions in regard to the action of certain diuretics when introduced directly into the renal artery to one kidney (the left kidney)

1 Novasurol, salyrgan and theocine-sodium-acetate when injected in small doses, directly into the left renal artery of the dog, produce a diuresis from the left kidney and little change in the excretory activity of the right kidney, in somewhat larger doses diuresis from each kidney varies but little, while when still larger doses are used there follows an active diuresis from the right and a not increased or a decreased excretion from the left kidney (the kidney into whose artery the diuretic is directly injected)

2 Caffeine citrate, theobromine-sodium-salicylate, theophylline-ethylene-diamine, theophylline-calcium-salicylate, urea and digitan in contrast, produce approximately the same urinary excretion from each kidney, although the diuretic has been injected directly into the renal artery of only one kidney

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# THE EFFECT OF THE HEART'S POSITION ON THE ELECTROCARDIOGRAPHIC APPEARANCE OF VENTRICULAR EXTRASYSTOLES<sup>1</sup>

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Within recent years the "classical" interpretation of electrocardiograms of bundle branch block, which was based on Lewis's work on dogs (1), has been questioned. Wilson and his coworkers (2) came to the conclusion that the bundle branch actually involved was just the reverse of the "classical" interpretation. These conclusions were based on the relative time of initial negativity of the two ventricles in man in bundle branch block as presumably given by so-called "semi-direct" leads from the chest wall over the right and left ventricles. The criterion used in such measurements, namely the point in the QRS group of the electrocardiogram where the deflection first begins to rise, has not been shown conclusively to indicate the activation of the region beneath the electrode. The second line of evidence is based on the appearance in ordinary indirect leads of extrasystoles produced experimentally in a clinical case of pericardial fistulae and pericarditis (Barker, Macleod and Alexander (3)) recently confirmed on another subject by Marvin and Oughterson (4). The direction of the QRS group of the extrasystoles from the two ventricles was just the reverse of that anticipated on the classical theory of Lewis. The evidence, however, is not convincing that these hearts were normal in size and position.

Some years ago Fahr (5) claimed to have shown on theoretical grounds that the classical interpretation of bundle branch block reversed the bundle branches. It has furthermore been known for a long time that while right bundle branch block was the more common electrocardiographic diagnosis, yet at autopsy left bundle branch block was more common (cf. Taussig (6)). It must be pointed out that the electrocardiographic and autopsy findings in individual cases do not always agree—in some cases they do, (Hill (8), Taussig (6)), in others they do not, (Oppenheimer and Pardee (7), Mahan (14)). The problem is further complicated by the fact that the electrocardiographic evidence of bundle branch block can be present without any demonstrable anatomical lesion.

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The foregoing argument concerning bundle branch block would apply with equal weight to extrasystoles of ventricular origin

Recently, in reviewing the subject of bundle branch block one of us, (Katz (9)), came to the conclusion that some of the difficulty involved in interpreting the electrocardiogram in bundle branch block was due to variations in the position of the heart in the chest and in the amount and distribution of ventricular hypertrophy and dilatation. Evidence has been presented on the isolated preparation, (Boden and Neukirch (10)), in the open-chested dog, (Meek and Wilson (11)), and in man, (Nathanson (12)), that shifting the heart's position altered the direction and amplitude of the major initial deflection in the electrocardiogram. The changes were for the most part those anticipated, except when the heart was rotated on its own long axis.

In the present study an attempt was made to analyze the changes, produced in different positions of the heart, in the electrocardiographic appearance of ventricular extrasystoles, derived from fixed points in the dog's ventricle.

#### EXPERIMENTAL METHODS

The experiments were made on seven dogs, anesthetized with barbital, with open chest and artificial respiration. Pairs of platinum electrodes were inserted in four regions of the heart, to wit, the base of the right ventricle, the apex of the right ventricle, the base of the left ventricle and the apex of the left ventricle. The wires from these electrodes were coiled and suspended from an insulated rod. By means of a distributor the current for producing the extrasystoles was sent at will to any of these regions. A modified Lewis rotating current interrupter was employed to induce the extrasystoles. The rate of these stimuli was adjusted so that an extrasystole was induced every third or fifth beat at approximately the same point in diastole. In three of the experiments the heart rate was controlled by another set of commutators connected to the same rotating pole that had the commutators for the extrasystoles. In this fashion a constant exact placement of the extrasystole in the heart cycle was obtained. It was found, however, that this refinement was not necessary if the extrasystoles were placed late in diastole.

The position of the heart was adjusted by proper traction on three threads sewn into the heart, one in the apex, one on the lateral wall of the left ventricle, and one on the lateral wall of the right ventricle. Six positions of the heart were used, viz:

- 1 Heart horizontal and apex pointing caudad<sup>2</sup>
- 2 Heart's apex up to form an angle of approximately 50° with the long axis of the body, apex pointing caudad
- 3 Heart's apex up 25°, and to the left 30° of the long axis of the body
- 4 Heart's apex up 25°, and to the right 30° of the long axis of the body
- 5 Heart's apex up 25°, to the left 15° of the long axis of the body, and heart rotated on its own long axis to bring right ventricle more anteriorly
- 6 Heart's apex up 25°, to the right 15° of the long axis of the body, and heart rotated on its own long axis to bring left ventricle more anteriorly

While it was attempted to make the shifts as simple as possible, displace-

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<sup>2</sup> The normal position of the heart's apex is 25° up from the horizontal

ments in other planes and rotation in moving the heart between positions in the first two pairs of positions (1 and 2, 3 and 4) could not be avoided.

The electrocardiograms, usual leads, were taken in each of these positions in quick succession in the following order. Lead I was taken with the distributor arranged to give extrasystoles first in the base of the right ventricle, then in the apex of the right ventricle, then in the base of the left ventricle, and then in the apex of the left ventricle. Leads II and III were taken with extrasystoles produced in the same order. Each record was standardized.

The amplitude of the major initial deflection in each record was measured and corrected for standardization. When a P wave fused with the QRS group, this was taken into account, to avoid error with large P waves. A positive major initial deflection was designated by plus, a negative one by minus. When the two phases were of almost equal amplitude they were both measured and placed with proper sign one after the other. These measurements are assembled in Table I. The change in the so-called electrical axis associated with the shift in the heart's position was determined by using Finthoven's vector analysis. The shift in electrical axis was determined between each pair of heart's positions, viz. positions 1 and 2, positions 3 and 4, and positions 5 and 6 above. This was facilitated by means of the twelve diagrams in Figure 1. Since it has been shown by Zeisler (13) that Einthoven's vector is only a rough estimate, and since the three leads were not taken simultaneously, nor are the peaks of the major deflections in the three leads necessarily homologous points, this analysis is to be considered no more than a rough estimate of the changes in the manifest potential. Nevertheless, as will be shown below, striking changes were found.

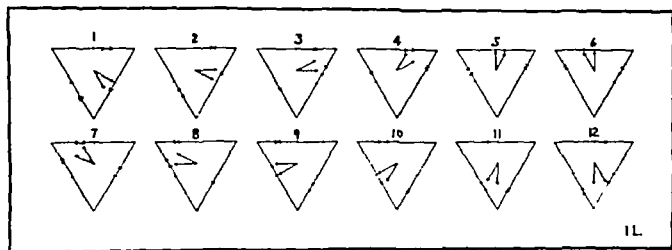


FIG. 1. DIAGRAM OF VECTOR ANALYSIS OF THE "MANIFEST POTENTIAL" ON FINTHOVEN'S TRIANGLE WITH THE "MANIFEST POTENTIAL" SHIFTED AROUND  $360^\circ$  IN TWELVE STAGES.

The arrows on the three sides of the triangle give the direction and relative magnitude of the projection of the "manifest potential" in each lead. As pointed out in the text, Einthoven's analysis is only a rough estimate.

#### RESULTS

It was found that as the apex of the heart was raised  $50^\circ$  from the horizontal, the amplitude of the major initial deflection of the sinus and extrasystolic beats decreased (cf. Table I and the complete protocol of Experiment 8 reassembled in Figures 2, 3, 4 and 5 to show the variation in configuration of the sinus beats and of the same extrasystoles in the



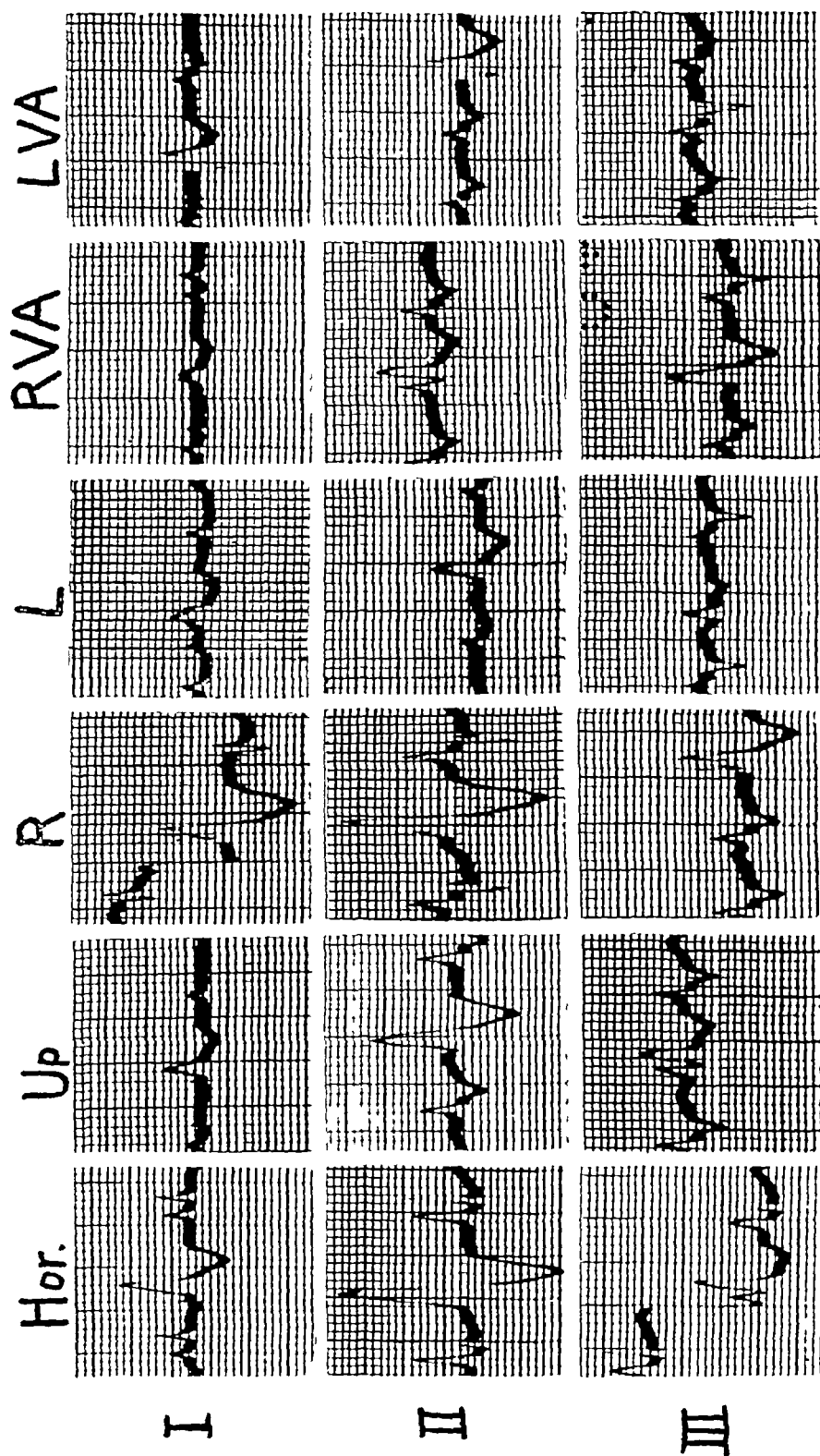


FIG. 2 THE ELECTROCARDIOGRAPHIC APPEARANCE IN THE ORDINARY THREE LEADS OF THE SINUS BEAT AND OF THE EXTRA-SYSTOLE INDUCED IN A FIXED REGION OF THE RIGHT VENTRICULAR BASE IN THE SIX POSITIONS OF THE HEART (EXPERIMENT 8)

*Hor* indicates heart apex pointing caudad *Up* indicates heart apex up so that heart forms an angle of  $50^\circ$  with the long axis of body *R*, heart apex to right of long axis of body so that heart forms an angle of  $30^\circ$  with it *L*, similar position to left of long axis of body *R V A*, heart apex to left of long axis of body so that heart forms an angle of  $15^\circ$  with long axis and at the same time heart rotated on its own long axis so as to bring the right ventricle anteriorly *L V A*, similar position to right of long axis of body and at the same time the heart rotated on its own long axis so as to bring the left ventricle more anteriorly

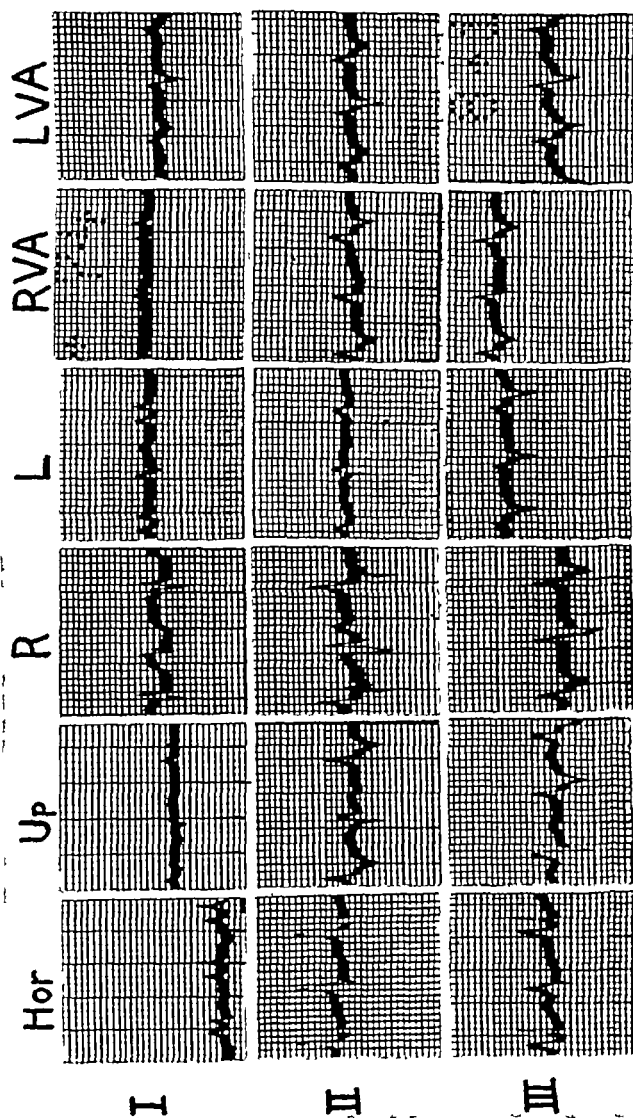


FIG. 3. THE ELECTROCARDIOGRAPHIC APPEARANCE IN THE ORDINARY THREE LEADS OF THE SINUS BEAT AND OF THE EXTRA-SYSTOLE INDUCED IN A FIXED REGION OF THE RIGHT VENTRICULAR APEX IN THE SIX POSITIONS OF THE HEART (EXPERIMENT 8). The positions of the heart are labelled as in Figure 2.

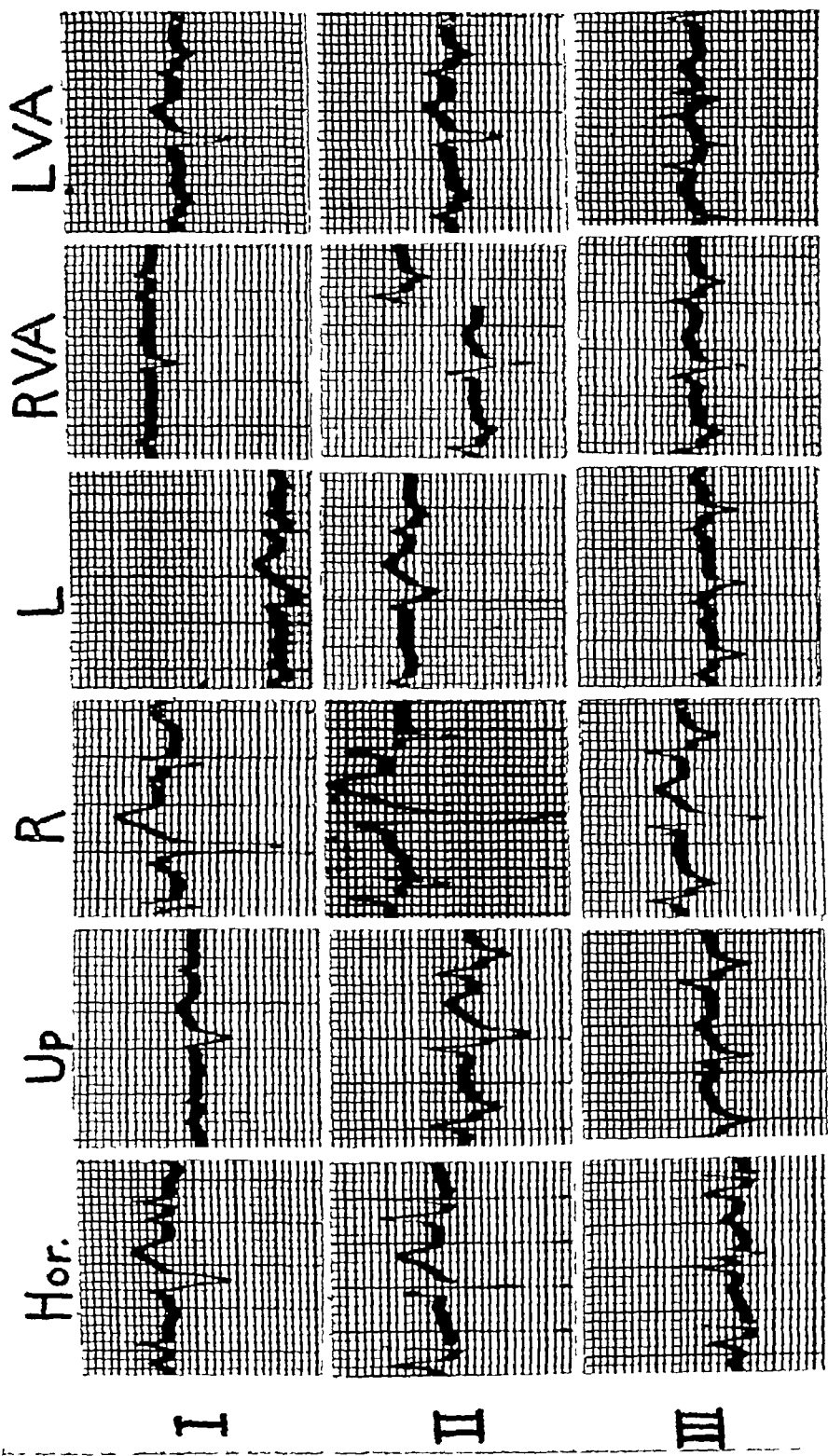


FIG 4 THE ELECTROCARDIOGRAPHIC APPEARANCE IN THE ORDINARY THREE LEADS OF THE SINUS BEAT AND OF THE EXTRASYSTOLE INDUCED IN A FIXED REGION OF THE LEFT VENTRICULAR BASE IN THE SIX POSITIONS OF THE HEART (EXPERIMENT 8)  
The positions of the heart are labelled as in Figure 2

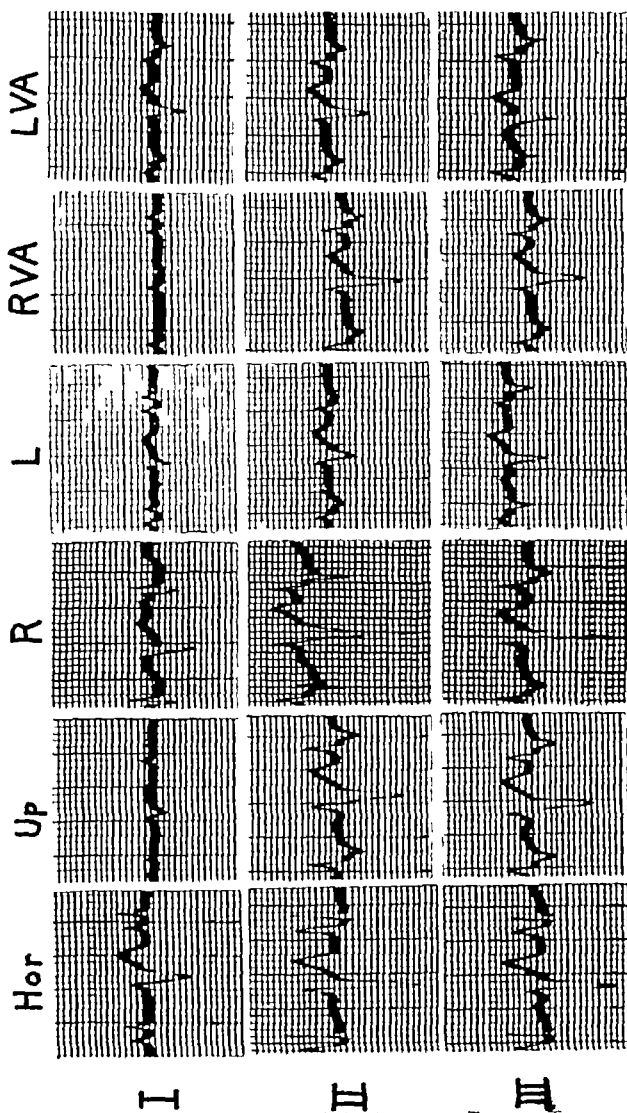


FIG. 5. THE ELECTROCARDIOGRAPHIC APPEARANCE IN THE ORDINARY THREE LEADS OF THE SINUS BEAT AND OF THE EXTRA SYSTOLE INDUCED IN A FIXED REGION OF THE LEFT VENTRICULAR APEX IN THE SIX POSITIONS OF THE HEART (EXPERIMENT 8). The positions of the heart are tab. II, 2, as in Figure 2.

## VENTRICULAR EXTRASYSTOLES

TABLE I

*Changes in the amplitude of the major initial deflection of sinus and extrasystolic beats with shifts in the heart's position \**

Experi- ment number	Lead	Heart hori- zontal	Heart apex 50° up from long axis of body	Heart apex 30° left of the long axis of body	Heart apex 30° right of the long axis of body	Heart apex 15° left of long axis of body and heart ro- tated on its own long axis to bring right ventricle more anteriorly	Heart apex 15° right of long axis of body and heart ro- tated on its own long axis to bring left ventricle more anteriorly
1 N	I	+ 1 5	+1	+ 6 5	- 1	- 1	+ 2 5
	II	+13	-1+1	+ 4	+17	+ 2 5	+ 6 5
	III	+12	-1	- 3	+15 5	+13 5	+ 6
1 RB	I	+ 6	+3 5	+13	+ 3 5	+ 4	+ 4 5
	II		+6	+ 6	+15	+ 5 5	+ 6
	III		-2	- 8	+11	+ 5 5	+ 3
1 RA	I		+2 5	+13	+ 3	+ 3	+ 4
	II	+ 6	+2	+ 6	+17	+ 3 5	+ 6
	III	+ 4	-1	- 8	+11	+ 4	- 3 5
1 LB	I		-3	-13 5	- 7	- 5 5	- 1
	II	- 3 5	+3	+ 4	+ 4-5	- 2 5	-12
	III	+10	+5 5	+10	+ 9	+ 9	+14
1 LA	I	- 3 5	-1	- 3	+ 7	- 2	- 1
	II	- 2+3 5	-2	- 4	+10	+ 2	- 1
	III	+ 6	-1	+ 3	+13	+11	+ 3 5
3 \	I	- 5+6	+5	+ 2	- 8 5+7		
	II	+17	+4	+18	+24		
	III	+19	+1 5	+ 7 5	+13		
3 RB	I	+ 6		+ 4 5	+ 9		
	II	+28		+18 5	+28 5		
	III	+21	+4	+ 7 5	+18 5		
3 RA	I			+ 2	- 5		
	II			- 9	-13		
	III			- 3	-13		
3 LB	I			-10	-10		
	II			-28 5	-28 5		
	III			-13	-13		
3 LA	I			-11 5	-11 5		
	II			-24	-24		
	III			-14	-14		

\* N = Normal complex

RB = Extrasystole from right base of heart

RA = Extrasystole from right apex of heart

LB = Extrasystole from left base of heart

LA = Extrasystole from left apex of heart

+ indicates an upright deflection

- indicates an inverted deflection

When a diphasic QRS is present the amplitudes of both phases are given, in order of their appearance

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TABLE I (continued)

Experiment number	Lead	Heart horizontal	Heart apex 50° up from long axis of body	Heart apex 30° left of the long axis of body	Heart apex 30° right of the long axis of body	Heart apex 15 left of long axis of body and heart rotated on its own long axis to bring right ventricle more anteriorly	Heart right axis and lateral to be ventricle
4 N	I	+ 8	-2+2	+13	- 2+2	+17 5	-
	II	+ 8 5	-2	+ 7 5	+ 6	+ 4	-
	III	- 4	-4 5	- 8	+ 6	-12 5	-
4 RB	I	+ 6 5	+2	+16	+ 5		
	II	+ 7	+2	+14	+11		
	III	- 3	+2 5	-10	+ 5		
4 LB	I	+ 5	+4				
	II	+ 2	+4				
	III	+ 5 5	+5				
4 LA	I	- 2	-0 5				
	II	- 8 5	-4				
	III	- 4	-9				
6 N	I	+ 1	+0 5	+ 1	- 0 5	+ 0 5	+
	II	+ 9	+1	+ 0 5	+ 0 5	+ 2 5	-
	III	+ 6 5	-2 5	- 3	+ 4 0	+ 4 5	-
6 RB	I	+ 3 5	+2	+ 2	+ 4 5	+ 2	+
	II	+ 4	+1	+ 1	+ 6	+ 4	-
	III	- 4	-5	- 4	+ 5	+ 9	-
6 RA	I	+ 4	+2	+ 2	+ 3	- 1	+
	II	+ 5	+1	+ 1	+ 5	+ 3 5	-
	III	- 7	-3	- 3	- 5	+10 5	-
6 LB	I	- 4	-2	- 2	- 4 5	- 3 5	-
	II	+ 8 5	+2 5	+ 1	- 4 5	- 2 5	+
	III	+11	+5 5	+ 2	+ 8	+10	+
6 LA	I	+ 1	+0 5	- 0 5	- 1	+ 2 5	-
	II	-16 5	-3	- 4	- 6	- 2	-
	III	-15	-6 5	- 8	-16	-12	-
7 N	I	+ 2	+1 5	+ 3	+ 2	+ 8 5	+
	II	+ 9	+7	+ 8	+14	+ 9	+
	III	+14	+8	+ 5	+13	+ 6	+
7 RB	I	+ 3 5	+2 5	+ 4 5	+10	+ 4 5	+
	II	+ 6 5	+6 5	+ 7 5	+26	+14	+
	III	+ 8 5	+6 5	+ 4 5	+14	+12	-
7 RA	I	+ 4	+2 5	+ 5 5	+10	+ 8	+
	II	+ 5	+3 5	+ 3	+13	+10	-
	III	- 6	-1 5	- 6 5	+ 4	+ 3	-
7 LB	I	- 3	-4	- 7	- 9	- 8 5	-1
	II	- 6 5	-7	- 6	-19	- 6	-1
	III	- 3	-3	+ 5	-11	+ 8 5	+
7 LA	I	- 1	-0 5	- 1 5	- 5	- 5 5	-
	II	- 4 5	-7	- 7 5	-19	-11 5	-1
	III	- 8	-6	- 4	-13	- 8	-

TABLE I (continued)

Experi- ment number	Lead	Heart hor- izontal	Heart apex 50° up from long axis of body	Heart apex 50° left of the long axis of body	Heart apex 30° right of the long axis of body	Heart apex 15° left of long axis of body and heart ro- tated on its own long axis to bring right ventricle more anteriorly	Heart apex 15° right of long axis of body and heart ro- tated on its own long axis to bring left ventricle more anteriorly
8 N	I	+ 2	+0.5	+ 0.5	- 2.5+3.5	+ 1	- 1
	II	+ 6	-2.5	- 2	- 5	- 2	- 1
	III	+ 4	-2	- 4	- 1.5	- 2	- 3
8 RB	I	+ 4	+2.5	+ 3	+ 8	+ 1.5	+ 3
	II	+12	+9	+ 5	+ 9	+ 7	+ 4
	III	+ 6	+3.5	+ 2	+ 7	+ 7	- 4
8 RA	I	+ 1	-0.5	- 1	- 2.5	+ 1	- 3
	II	- 1	-4	- 2.5	- 7	+ 3	- 5
	III	- 1	-3.5	- 3.5	- 3.5	+ 2	- 3
8 LB	I	- 3.5	-2	- 4	-11.5	+17.5	- 7
	II	- 6.5	-4.5	- 3.5	-22	+ 4	- 6
	III	- 2	-3	+ 0.5	-10	-12.5	- 2
8 LA	I	- 2.5	-0.5	- 2	- 6	+ 0.5	- 4
	II	- 9	-8	- 3.5	-18	- 8	- 6
	III	- 7	-7	- 4.5	-11	- 8	- 6
9 N	I			+ 7	- 2.5		
	II			+ 7	+ 7		
	III			- 3.5	+ 7		
9 RA	I			+10	+12		
	II			+11	+16		
	III			+ 2-2.5	+ 9		

various positions of the heart) The variability in the shift of the electrical axis to the right or left is shown in Table II. The shifts of the electrical axes of the various extrasystoles in a single experiment were not proportional to each other nor to the shift in the electrical axis of the sinus beat. In fact, in practically every one of the experiments the shift in electrical axis was in an opposite direction in some beats to that in others of different origin.

Usually the deflections were larger when the apex of the heart was to the right of the long axis of the body than when the apex was to the left. The deflections were also larger when the right ventricle was rotated anteriorly than when the left ventricle was rotated anteriorly, although there were many exceptions (Table I).

It was found that moving the heart's apex from right to left in relation to the long axis of the body with or without rotation of the heart on its own long axis (viz., positions 3 and 4, 5 and 6) also gave a lack of proportionality in amount and a discordancy in direction of the shift of the electrical axis of the various types of beats (Table II). Furthermore,

TABLE II  
*Shift in electrical axis*

Experiment number	Mechanism*	Shift of heart from horizontal up	Shift of heart from right to left	Shift of heart from right to left plus rotation of heart on its own long axis from left ventricle anterior to right ventricle anterior
1	Normal RB RA LB LA	Left Left Left  Right	Left Left Left Right Right	Right Left Right Left Right
3	Normal RB RA LB LA	Left	Left Left Right Left None	
4	Normal RB LB LA	Left Right  Right	Left Left	Right
6	Normal RB RA LB LA	Left None Right Left Right	Left Left None Left Left	Right Right Right Right Right
7	Normal RB RA LB LA	Left None Right None Left	Left Left Left Left	Left Right Right Left None
8	Normal RB RA LB LA	Left None Left Right Right	Right None Right Left Left	Right Right Right (180°) Right Right
9	Normal RA		Left Left	

\* Normal equals sinus rhythm

RB = Extrasystole from right base

RA = Extrasystole from right apex

LB = Extrasystole from left base

LA = Extrasystole from left apex

while the shift of the electrical axis was in the same direction as the shift in the anatomic axis in most types of beats when the heart's apex was moved from right to left of the long axis of the body with practically no rotation of the heart on its own long axis the shift of the electrical axis



was opposite in direction to that of the anatomic shift in most types of beats when this movement of the heart's apex was simultaneous with rotation of the heart on its own long axis (Table II). This result agreed with the observations of Boden and Neukirch (10), Meek and Wilson (11), and Nathanson (12) on the sinus beats. We have not only confirmed these results but have shown that they apply also to ectopic beats arising in various parts of the ventricle.

The variations in the shift of the electrical axes gave rise, as might have been expected, to reversal in the direction of the major deflection of the QRS group in a number of instances. The frequency of this occurrence has been summarized in Tables III and IV and typical examples are

TABLE III

*On shifting heart's anatomic axis from right to left following reversals of direction of QRS noted*

4 out of 7 N	shifted	- to +	Lead I
4 out of 7 N	shifted	+ to -	Lead III
3 out of 6 RB	shifted	+ to -	Lead III
1 out of 6 RA	shifted	- to +	Lead I
2 out of 6 RA	shifted	+ to -	Lead III
1 out of 5 LA	shifted	+ to -	Leads I and II
1 out of 5 LB	shifted	- to +	Lead II
2 out of 5 LB	shifted	- to +	Lead III

N = Normal complex

RB = Extrasystole from right base of heart

RA = Extrasystole from right apex of heart

LB = Extrasystole from left base of heart

LA = Extrasystole from left apex of heart

TABLE IV

*On shifting heart's anatomic axis from right to left and rotation on long axis from left ventricle anterior to right ventricle anterior following reversals of direction of QRS noted*

1 out of 5 N	shifted	- to +	Leads I and III
1 out of 5 N	shifted	+ to -	Lead I
2 out of 5 N	shifted	- to +	Lead II
1 out of 4 RB	shifted	- to +	Lead II
3 out of 4 RB	shifted	- to +	Lead III
1 out of 4 RA	shifted	- to +	Lead I
1 out of 4 RA	shifted	+ to -	Lead I
3 out of 4 RA	shifted	- to +	Lead II
4 out of 4 RA	shifted	- to +	Lead III
1 out of 4 LB	shifted	- to +	Leads I and II
1 out of 4 LB	shifted	+ to -	Lead II
2 out of 4 LA	shifted	- to +	Lead I
1 out of 4 LA	shifted	- to +	Lead II

N = Normal complex

RB = Extrasystole from right base of heart

RA = Extrasystole from right apex of heart

LB = Extrasystole from left base of heart

LA = Extrasystole from left apex of heart

shown in Figures 6, 7 and 8, as well as in Figure 2 (cf. Lead III of RVA and LVA). Thus in Figure 6, when the heart's apex is shifted from the right to the left of the long axis of the body, the major initial deflection of the extrasystole increases in Lead I, decreases in Lead II and becomes inverted in Lead III, the QRS group of the sinus beat changes from a small inverted to an upright deflection in Lead I, decreases in size in Lead II, and becomes inverted in Lead III. In Figure 7 the major

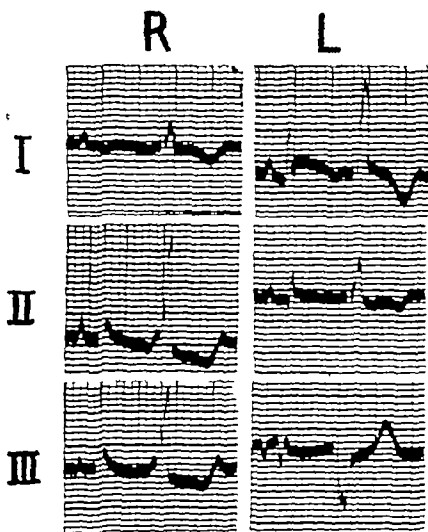


FIG. 6 THE ELECTROCARDIOGRAPHIC APPEARANCE IN THE ORDINARY THREE LEADS OF THE SINUS BEAT AND OF THE EXTRASYSTOLE INDUCED IN A FIXED REGION OF THE RIGHT VENTRICULAR APEX IN TWO POSITIONS OF HEART (EXPERIMENT 1)

R and L have the same significance as in Figure 2. Note reversal of direction of QRS of the extrasystole in Lead III.

initial deflection of the extrasystole increases in size, especially in Lead II, while in Lead III it becomes inverted on shifting the heart's apex from the left to the right of the body's long axis. At the same time the sinus beat became more upright in all leads. In Figure 8 the changes are particularly striking since the major initial deflection is reversed in the two positions in Leads II and III, indicating a shift in the electrical axis of approximately  $180^\circ$  in the plane of the leads. The sinus beat shows a similar shift in the direction of the QRS group.

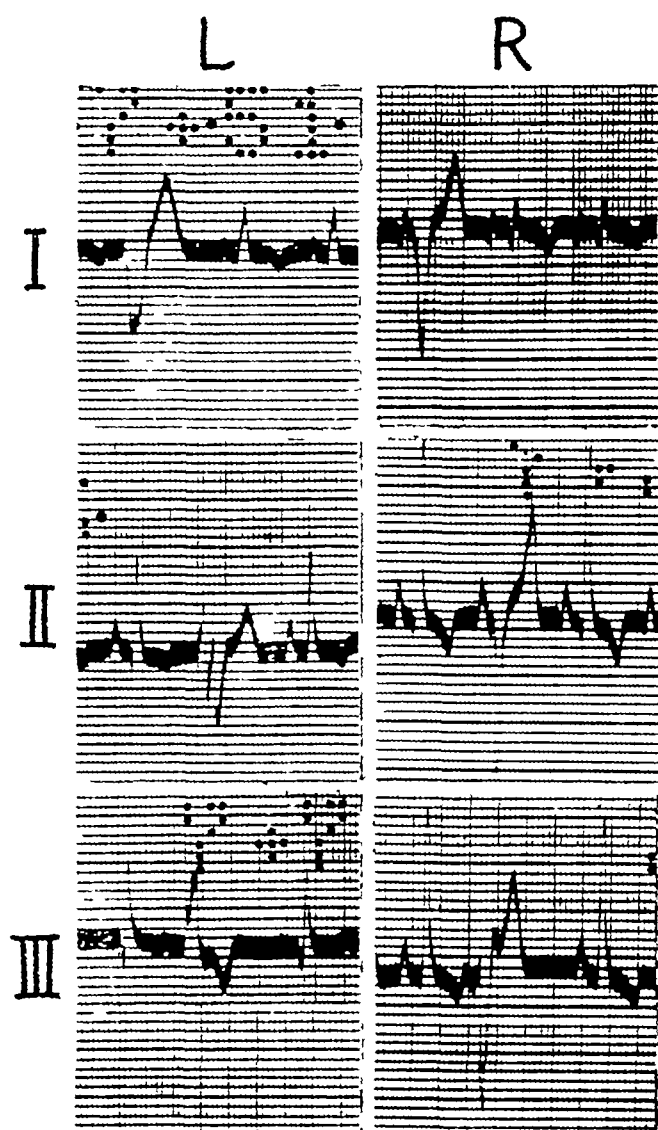


FIG 7 THE APPEARANCE OF THE SINUS BEAT AND OF THE EXTRASYSTOLE INDUCED IN A FIXED REGION OF THE LEFT VENTRICULAR BASE IN TWO POSITIONS OF HEART (EXPERIMENT 7)

R and L of same significance as in Fig 2 Note reversal of QRS of the extrasystole in Lead III

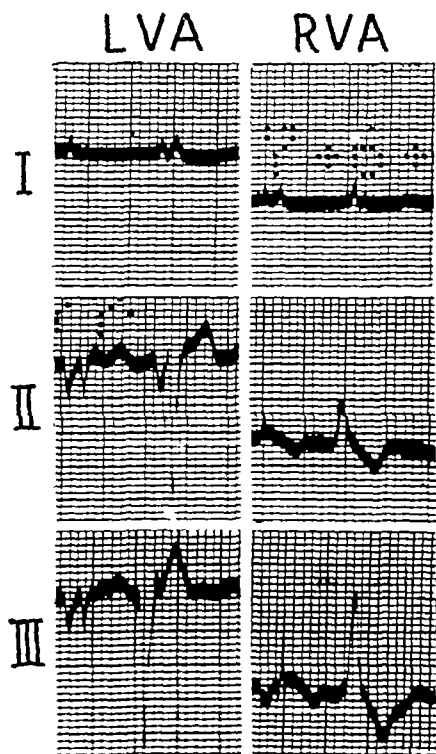


FIG 8 THE ELECTROCARDIOGRAPHIC APPEARANCE IN THE ORDINARY THREE LEADS OF THE SINUS BEAT AND OF THE EXTRASYSTOLE INDUCED IN A FIXED REGION OF THE RIGHT VENTRICULAR APEX (EXPERIMENT 6)

RVA and LVA of same significance as in Fig 2 Note reversal of QRS in Leads II and III

## DISCUSSION

The lack of proportionality of the changes, and the shift in opposite directions in the electrical axis of the various extrasystoles in a single experiment shows that the shift in the anatomic axis is not the only factor involved. The disparity arises from the fact that potentials set up by these extrasystoles act in three dimensions. Changes in the position of the heart therefore will change the projection of the vector in three dimensions cast on the plane formed by the three leads.<sup>3</sup>

The projection of this "three-dimensional" vector on the plane of the three leads varies monotonically with the inverse of the angle which it forms with this plane. When the position of the heart is changed, the angles between the three-dimensional vectors of the extrasystoles and the plane of the three leads are altered unequally and sometimes in such a way that the projections on this plane move in opposite directions, as can be seen from the diagram of Figure 9. This is particularly important

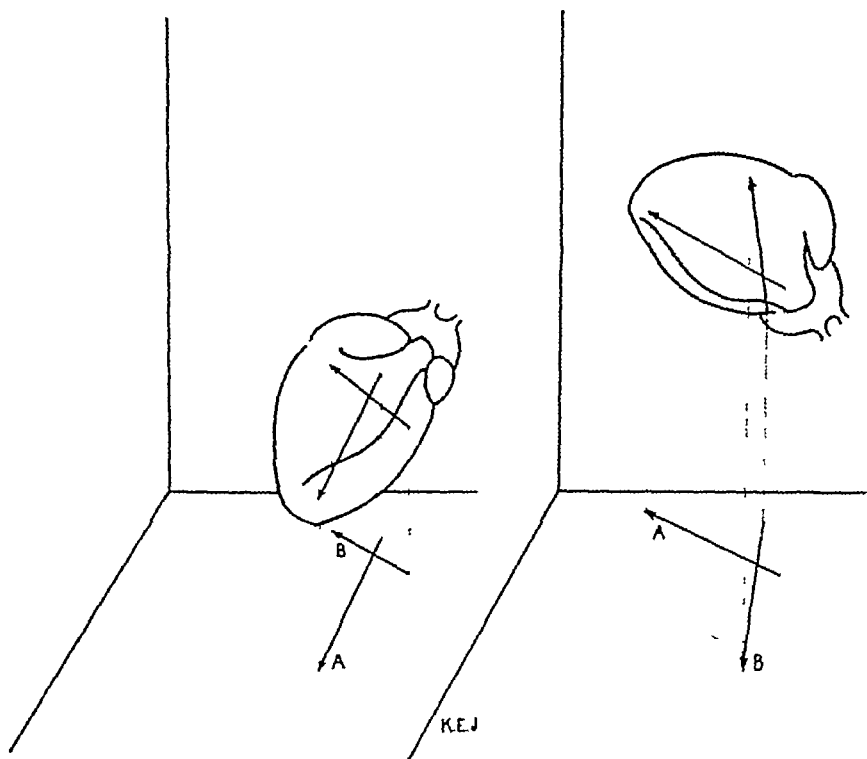


FIG 9 THREE-DIMENSIONAL DIAGRAM SHOWING THE MOVEMENT IN OPPOSITE DIRECTIONS OF THE PROJECTION ON THE PLANE OF THREE LEADS OF THE THREE-DIMENSIONAL VECTORS PRODUCED BY TWO EXTRASYSTOLES WHEN THE POSITION OF THE HEART WAS CHANGED

A and B are the projections of the two vectors on the plane of leads

<sup>3</sup> The projection on the plane of the three leads of the vector in three dimensions has been termed the electrical axis of the heart in this paper, since it is commonly so used

since rotation of the heart on its own long axis has such a tremendous influence, and since it is impossible to move the heart without changing the relation of the chambers to the plane of the leads

In other words, we can view the movement of the heart in three dimensions as bringing into prominence the effect of new regions and decreasing the effect of the old ones. This may bring about a new balance in the plane of the leads in a direction opposite to the shift in the projection of the anatomic axis in this plane. Results such as presented in this report reemphasize the fact that the three standard leads constitute only two dimensions of the three dimensional potential differences set up by the heart.

The ability, by shifting the position of the heart, to reverse the direction of the major initial complex of ventricular extrasystoles evoked from fixed spots shows that the position of the heart and its configuration cannot be ignored in analyzing the origin of extrasystoles and the location of bundle branch block. Furthermore, these results would tend to show that the configuration of the electrocardiograms of the extrasystoles induced in the human heart by Barker, Macleod and Alexander (3) and by Marvin and Oughterson (4) may have been distorted by rotation and change in position of the heart or by dilatation of the ventricular chambers, and it would be hazardous to assume without further proof that their results can be applied to the normal human heart.

Although the changes observed in our experiments are probably more marked than occur in man they are nevertheless applicable since rotation of the heart on its own long axis has the greatest influence on the electrical axis. Hypertrophy and dilatation by changing the relation of the heart's chambers to each other probably have the same influence as rotation of the heart on its axis, and such changes are probably as marked as those produced by these rotations. In favor of this view is the fact that deviations just as marked as in our experiments sometimes occur in the electrical axis of the sinus beats in human hearts.

The results confirm the impression previously stated, that changes in the heart's position resulting from displacement or from dilatation and hypertrophy of the ventricles may be responsible in many instances for the discrepancy between the electrocardiographic and postmortem diagnosis of bundle branch block. The frequency of preponderant left ventricular hypertrophy can explain the inversion of the major initial deflection in Lead III. In other words, the bundle branch block increases the duration of the QRS group and the preponderant hypertrophy determines the direction of the major deflections.

In the present state of knowledge and with the variability in direction of the QRS group which these experiments show can be produced by changing the position of the heart, it would be preferable not to attempt to locate the site of origin of ventricular extrasystoles and, for the same

reason, bundle branch block. Instead, the diagnosis should be given as follows: intraventricular block of the so-called bundle branch block type.

We have found a similar result with experimentally produced bundle branch block (Ackerman and Katz (15)).

#### SUMMARY

1. Studies were made of the effect, in the open-chested anaesthetized dog, of changing the heart's position on the electrocardiograms of induced extrasystoles. The shifts of the heart were (a) in an anteroposterior direction on the transverse axis at its base, (b) in a lateral direction on the anteroposterior axis at its base with practically no rotation on its long axis, and (c) rotation on its long axis (which runs from the base to apex) with some movement in a lateral direction on the anteroposterior axis at its base. The extrasystoles were induced in four regions, viz., the base and apex of the right and left ventricles.

2. The electrical axis of the various extrasystoles did not move the same amount nor always in the same direction when the heart was shifted. The electrical axis moved in the same direction as the anatomic axis in most types of beats when practically no rotation on the heart's own long axis accompanied the change in the heart's position. The electrical axis moved in a direction opposite to the anatomic axis in most types of beats when the heart was rotated on its own long axis at the same time.

3. Many times the direction of the major initial deflection of the extrasystoles was reversed in one or more leads.

4. The results are attributed to the unequal movements of the so-called three-dimensional vectors of the various extrasystoles when the heart was shifted. This resulted in an unequal and at times opposite movement of the projections of these three-dimensional vectors on the plane of the three leads.

5. The reversal in direction of the QRS group of ventricular extrasystoles, which often accompanied changes in the heart's position, shows (a) that the configuration and position of the heart cannot be ignored in analyzing the site of ventricular extrasystoles and bundle branch block in man, and (b) that the direction of the QRS group of extrasystoles induced in the two patients reported with pericarditis and pericardial fistula may not be applied to the non-diseased human heart without consideration of the other factors involved.

6. On the basis of these results it would be unjustified to attempt to localize the site of ventricular extrasystoles or of bundle branch block from the direction of the major initial complex in the three leads of their electrocardiograms.

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# STUDIES ON THE ELECTRICAL SYSTOLE ("Q-T" INTERVAL) OF THE HEART

## IV THE EFFECT OF DIGITALIS ON ITS DURATION IN CARDIAC FAILURE

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Though digitalis has been known in medicine since 1785 and its beneficial effects have been intensively studied, its action on the heart muscle is still far from fully understood. Numerous studies have been made on animals, but very little is known of its action on the dynamics of the human heart. Many theories have been proposed to explain the beneficial results of its use in disease, but to a large extent these have been based on animal experimentation or inferred from clinical observation. The work here reported was undertaken in the hope of obtaining information concerning the mechanism of the action of digitalis on the human myocardium in disease. At the same time a better understanding of this process might be expected to throw important light on the mechanism of heart failure.

In a previous study it was found that the electrical systole ("Q-T" interval of the electrocardiogram) varies with the cycle length in a way which can be expressed sufficiently accurately by the formula,  $S = K \sqrt{C}$ , in which "S" is the "Q-T" interval, "C" the "R-R" interval of the electrocardiogram, and "K" has the value of  $0.374 \pm 0.0012$  for normal Chinese men and  $0.388 \pm 0.0015$  for normal Chinese women (1). It has also been shown that in patients with heart failure the "Q-T" interval is prolonged in relation to cycle length, so that "K" in the formula just given was increased in average value to  $0.432 \pm 0.0023$  for 121 men and  $0.432 \pm 0.0027$  for 100 women in our series of patients with myocardial insufficiency (2). It would seem that this finding constitutes an important factor in the dynamic disturbance under discussion.

For the present study<sup>1</sup> a large number of patients with heart failure have been observed. They have all been under our clinical direction. The cases presented were not in any way selected. Electrocardiograms were taken and measured by one of us under conditions described in the previous papers. Measurements were made from lead II with a few exceptions in which the "T" wave of lead II was indistinct (but the same

<sup>1</sup> A preliminary report of this work was published in 1931 (3), at which time the work was completed. Subsequently the article of Berliner (4) appeared.

TABLE 1

*Electrocardiographic measurements showing the effect of digitalis on "R-R" and "Q-T" intervals*

E K G number 2662 Female, age 14 Active rheumatic heart disease, mitral and aortic disease, pericarditis, heart failure IV

Date and hour	P-R interval	T 2	R-R interval	Q-T interval	K' *	Digitalis † Remarks	Calculated Q-T interval ‡	Calculated duration systole ‡	Actual duration systole
	seconds	mm	seconds	seconds		grams	seconds	seconds per minute	seconds per minute
January 20									
9 a m	16	3 5	540	330	449	None Weight 29 kgm	285	31 6	36 6
2 p m	16	3 5	535	335	450	None			
5 p m	16	4 0	530	325	447	0 3 at 5 and 8 p m	283	31 9	36 8
January 21									
9 a m	18	3 5	635	290	364	0 6 at 8 a m	309	29 2	27 4
2 p m	24	3 0	680	280	339	0 5 at 10 a m			
5 p m	20	2 0	640	255	319	0 2 at 1 p m			
January 22									
9 a m	20	3 0	590	240	318	Total 1 9	298	30 3	24 4
2 p m	24	0	936	278	287	Occasional 2 1 block			
5 p m	24	2 5	970	315	319				
January 23									
9 a m	24	2 5	695	230	276	Occasional 2 1 block	322	27 9	20 0
2 p m	24	2 0	690	274	330	Occasional 2 1 block			
5 p m	26	2 0	690	250	301				
January 24									
9 a m	24	2 0	710	240	280		327	27 6	20 2
3 p m	20	2 5	645	250	311				
5 p m	18	3 0	625	250	316				
January 26	20	3 0	620	245	311		305	29 5	23 7
January 29	20	3 5	630	270	340	Weight 26 kgm	308	29 3	25 8
February 4	16	4 0	650	300	372		313	28 8	27 6
February 12	16	5 0	635	325	408		309	29 1	30 7
March 2	16	5 0	520	320	443		280	32 3	36 9
March 9	16	5 5	540	320	435		285	31 6	35 5

\* "K" = "Q-T" interval  $\sqrt{\text{"R-R" interval}}$  Its average value for normal female Chinese is  $0.388 \pm 0.0015$ , for males  $0.374 \pm 0.0012$  (1)

† Digitalis was given by mouth in the form of compressed powdered leaves, assayed  $92 \pm 5.2$  mgm per cat unit.

‡ The "Q-T" interval calculated for the actual rate by using the average normal value of "K" Using the value obtained the duration of systole per minute is calculated for comparison with the actual duration

lead was always used in a given patient) A few instances of auricular fibrillation were included, in these cases an average of 16 "R-R" and "Q T" measurements was calculated Special attention was given to one or more records taken before the patient received digitalis, except in some cases included because observations were subsequently made after digitalis had been discontinued for a long period Digitalis was given by mouth as compressed powdered leaves, assayed to have a value of  $92 \pm 5.2$  mgm per cat unit No very precise rule for dosage was followed, but the majority of the patients were "digitalized" in 36 to 48 hours The usual clinical observations were carefully made, but are not presented as they are of no special interest In most of the patients the heart size was measured in teleoroentgenograms according to the method of Hodges and Eyster (5)

## RESULTS

In selected cases serial records were made for several days at the same hours before and after digitalis treatment which was pushed to the point at which a clinical effect was clearly seen Five such cases are summarized in Tables 1 to 5 The results in 45 males and 28 females studied in less

TABLE 2  
*The effect of digitalis on "R R" and "Q T" intervals*

E.K.G. number 3053 Male, age 21 Active rheumatic heart disease, mitral and aortic disease, pericarditis, heart failure III

Date and hour	P R interval	T 2	R R interval	Q-T interval	K *	Digitalis.*	Remarks
	<i>seconds</i>	<i>mm</i>	<i>seconds</i>	<i>seconds</i>			<i>grams</i>
November 19							
9.30 a.m.	16	3.8	.585	.360	470	None	Weight 44 kgm
2.30 p.m.	16	4.0	.555	.330	443	None	
5.30 p.m.	16	4.0	.545	.335	454	None	
November 20							
9.30 a.m.	16	4.0	.590	.320	417	1.4 in 15 hours	
2.30 p.m.	16	3.0	.605	.305	.392	2.2 in 20 hours	
5.30 p.m.	16	3.0	.610	.310	397	2.2 in 23 hours	
November 21							
9.30 a.m.	16	3.0	.600	.280	.361	2.8 in 39 hours	
2.30 p.m.	16	2.0	.475	.240	.348	3.2 in 44 hours	
5.30 p.m.	17	3.0	.520	.250	.342	No more	
November 22							
9.30 a.m.	18	3.0	.665	.242	297		
2.30 p.m.	.20	4.0	.615	.240	.306		
5.30 p.m.	18	4.0	.580	.240	.316		
November 24	16	4.0	.530	.265	.364	Weight 40 kgm	

\* See footnotes to Table 1

detail are given in Tables 6 and 7. We have continued to use the value of "K" (the ratio of systole to the square root of cycle length) as a convenient means of comparison.

TABLE 3  
*The effect of digitalis on "R-R" and "Q-T" intervals*

E K G number 3056 Male, age 61 Syphilis of cardiovascular system, aortic regurgitation, aneurysm of ascending aorta, heart failure, IIb

Date and hour	P-R interval	T 2	R-R interval	Q-T interval	' K *	Digitalis * Remarks
	<i>seconds</i>	<i>mm</i>	<i>seconds</i>	<i>seconds</i>		<i>grams</i>
November 18	16	3 0	890	420	445	None Weight 48 kgm
November 25	16	3 0	815	395	438	None
November 26						
10 a m	16	3 0	900	375	395	None
2 p m	16	3 2	640	345	432	None
5 p m	16	3 0	728	364	428	0.5 at 6 and 10 p m
November 27						
9 30 a m	16	3 0	977	425	431	0.5 at 9 a m
2 p m	16	3 0	900	420	444	0.3 at 1 p m
5 p m	16	3 0	880	423	451	0.2 at 8 p m
November 28						
9 a m	16	3 0	995	395	396	0.2 at 6 a m
2 p m	16	3 0	785	325	367	0.2 at 10 a m and 1 p m
5 p m	16	3 0	770	349	397	0.3 at 9 p m
November 29						
9 a m	16	3 0	940	360	372	Total 2.9
2 p m	16	3 0	965	385	392	
5 p m	16	3 0	955	378	384	
December 1	16	3 0	835	345	378	Weight 43 kgm
January 8	16	3 0	940	320	330	
January 23	15	3 0	940	360	371	

\* See footnotes to Table 1

Digitalis was found to shorten the relative length of the "Q-T" interval with remarkable consistency. The same finding has been reported by Berliner (4). We have previously reported the same result in normal persons (6). The shortening occurs at least as early as any other known effect of digitalis. It takes place simultaneously with the lowering of the "T" wave (7) and precedes a change of "T" to a diphasic or negative form and a sagging of the "Q-T" level (cf Tables 1 and 2). In some cases "T" remains unchanged, although systole is relatively shortened (cf Table 3). Some time after withdrawal of digitalis systole

returns to approximately its previous relative value, usually in parallel with the return of "T" to its former height, but occasionally "T" remains depressed for a longer period

That the amount of digitalis effective in producing the relative shortening of systole does not always follow the body weight is shown in

TABLE 4  
*The effect of digitalis on "R R" on "Q-T" intervals*

E K.G. number 3011 Male, age 41 Syphilis of cardiovascular system, aortic regurgitation heart failure III

Date and hours	P R Interval	R R Interval	"Q-T" Interval	K *	Digitalis * Remarks
	seconds	seconds	seconds		grams
November 4					
10 a.m	12	.505	288	405	None Weight 62 kgm
2.30 p.m	16	.526	288	.397	None
5 p.m	14	.524	.300	415	0.5 at 5.30 p.m 0.4 at 9.30 p.m
November 5					
9.30 a.m	16	.530	295	405	0.4 at 8 a.m
2.30 p.m	12	.505	280	399	0.4 at noon
5.30 p.m	14	.508	.255	.358	0.4 at 4 and 6 p.m
November 6					
8.30 a.m	20	.456	196	290	0.4 at 4 and 8 a.m
2.30 p.m	.20	.483	188	270	Total 3.3
5.30 p.m	?	.570	?	?	Auricular fibrillation
November 7					
9.30 a.m	?	.554	.253	340	Auricular fibrillation
3.30 p.m	.20	.570	251	332	Normal mechanism
November 8	28	.585	?	?	Normal mechanism

\* See footnotes to Table 1

Table 5A (data from Tables 1 to 5) In the cases shown in Tables 4 and 5 digitalis was pushed to a point at which auricular fibrillation occurred, as also happened with some of the cases in Tables 6 and 7 In these and in other instances of excessive digitalis administration the ratio of systole to the square root of cycle length ("K") was lowered often far below the usual normal value In the cases of Tables 4 and 5 "K" was 0.270 and 0.326 just before fibrillation set in It is our impression that reduction of "K" to or below 0.330 indicates the beginning of a toxic as opposed to a therapeutic effect. This may sometimes happen with a truly small dose as in Case 3478, Table 7, in which after 0.8 gram digitalis, "K" was 0.298, the pulse 50, and the "P-R" interval 0.40 second It should be noted that the original value of "K" in this case was only 0.366 The value of "K" has also appeared to furnish a guide to the dose of digitalis necessary to maintain a patient in his optimal condition, for which the usual clinical criteria are sometimes slow in developing and difficult to interpret

TABLE 5

*The effect of digitalis on "R-R" and "Q-T" intervals*

E K G number 3069 Female, age 30 Rheumatic heart disease, mitral stenosis, heart failure IIB

Date and hour	'P-R' interval	T 2	'R-R' interval	Q-T interval	'K' *	Digitalis.* Remarks
	seconds	mm	seconds	seconds		grams
December 3						
9 30 a m	16	2 0	510	260	365	None Weight 56 kgm
2 p m	16	2 0	525	270	374	None
5 p m	16	2 0	528	280	386	0.5 at 6 and 10 p m
December 4						
9 30 a m	16	2 0	580	275	363	0.5 at 8 a m and noon
2 p m	16	2 0	550	245	331	
5 p m	16	2 0	555	235	318	
December 5						
9 30 a m	16	2 5	660	253	314	0.2 at 6 a m, 0.4 at 10 a m
2 p m	16	2 5	765	304	350	
5 p m	?	3 0	608	258	334	Auricular fibrillation
December 6						
9 a m	?	2 0	597	230	300	Auricular fibrillation
2 p m	16	2 0	580	264	334	Normal mechanism
5 p m	16	2 0	605	250	324	Weight 50 kgm
December 11	16	2 0	620	275	352	
December 15	16	2 5	645	305	381	Weight 44 kgm

\* See footnotes to Table 1

The tables contain several examples of patients in whom the effect of digitalis on the relative length of systole was repeatedly brought out by alternating periods of withdrawal and administration of the drug. In some cases it appeared that a smaller dose was effective on a second or later occasion than was necessary at first. In Case 2677 (Table 6) digitalis 1.4 gram in 3 days brought the value of "K" from 0.416 to 0.403 and later after an interval of four weeks without digitalis, 1.5 gram in 3 days re-

TABLE 5A

*Comparison of body weight and effective dose of digitalis*

E.K.G number	Age	Sex	Weight	Effective dose digitalis		Time elapsed
	years		kgm	grams	grams per kgm	hours
2662	14	F	26	1.2	0.046	16
3053	21	M	40	1.8	0.045	17
3056	61	M	43	2.2	0.051	28
3069	30	F	43	1.5	0.035	15
3011	41	M	62	1.3	0.021	18

TABLE 6

*Effect of digitalis on the duration of the "Q-T" interval in 45 male Chinese with heart failure*

## ABBREVIATIONS

A D	= aortic disease (stenosis and regurgitation)	Cor Ob	= coronary obstruction
A F	= auricular fibrillation	G.A	= general arteriosclerosis
A.R	= aortic regurgitation	H	= hypertension
Ac Neph	= acute nephritis	M D	= mitral disease (stenosis and regurgitation)
Chr Neph	= chronic nephritis	P T b	= pulmonary tuberculosis.
		S	= syphilis

E.K.G num ber	Age	Clinical diagnosis	Date	Weight	"P R Interval	Heart rate	K' *	Digitalis†
	years			kgm.	seconds			grams
A	Rheumatic heart disease							
2019	26	M D	August 18	63	17	102	.384	1 0 in 24 hours
			August 21		16	97	.367	1 8 in 5 days
2201	33	M D, S	March 4		16	111	436	None
			March 11	58	.20	86	.396	1 5 in 7 days
2244	22	M D	January 14		17	93	402	None
			January 21	49	16	100	.374	1 5 in 5 days
			February 22		.20	105	.369	4 2 in 25 days
			March 11		20	48	.301	4 9 in 32 days
2340	22	A.D	March 29		.20	76	402	None
			April 1	55	20	72	.395	0 7 in 4 days
			April 5		20	73	.390	1 5 in 8 days
			April 14		.20	63	.375	3 2 in 17 days
			June 4		.20	81	431	None for 1 month
2344	30	M D	March 30		16	80	447	None
			April 1		18	78	409	0 6 in 2 days
2717	28	M D	March 20		13	123	414	None
			March 24	47	16	87	.380	1 7 in 5 days
2780	23	M D	May 2		20	63	408	None
		A D	July 25	46	20	65	.335	4 3 in 39 days
2865	41	M D, A.D	July 11		.28	95	438	None
			July 14	46	28	90	427	1 4 in 4 days
			November 12		.32	98	421	None for 1 month
			November 22		24	92	.398	0 8 in 12 hours
			December 6		.32	75	.380	3 0 in 16 days
2903	21	M.D, A.D	July 25		16	115	441	None
			July 30	58	16	99	360	2 0 in 6 days
			August 5		20	86	.346	2 6 in 12 days
			August 21		20	87	.385	None for 12 days



TABLE 6 (continued)

E.K.G. number	Age	Clinical diagnosis	Date	Weight	P-R interval	Heart rate	"K" *	Digitalis†
	years			kgm	seconds			grams
2909	36	M D	August 5	46	18	107	428	None
			August 8		24	81	382	1 6 in 4 days
			August 16		27	56	320	2 8 in 12 days
			September 26		20	101	415	None for 21 days
			October 6		25	81	366	1 9 in 10 days
			October 20		24	87	349	3 2 in 23 days
3162	38	M D	March 2		18	110	409	None
			March 6		19	102	390	1 5 in 4 days
			March 13		22	82	357	2 3 in 11 days
			March 23		24	78	360	3 5 in 20 days
			March 27		20	87	372	3 9 in 24 days
3293	17	M D, P T b	June 15	30	16	98	423	None
			June 22		16	84	331	1 0 in 24 hours
			June 25		16	78	320	1 4 in 48 hours
<i>B Syphilitic heart disease</i>								
2033	45	S, Tabes, H	March 28	54	16	83	412	None
			April 3		16	81	395	1 2 in 7 days
			July 18		16	87	379	0 1 q d
2054	38	S, A R	September 17	68	19	114	373	1 5 in 2 days
			September 21	59	20	96	370	1 9 in 6 days
			October 3		19	100	411	None for 5 days
2055	60	S, A R	September 17		16	80	360	1 7 in 10 days
			October 1		17	80	353	0 1 q d
			November 19		16	80	480	None for 14 days
			December 18		16	68	434	0 1 q d
			April 22		16	59	369	2 0 in 7 days
			May 18		18	64	497	None for 4 months
			June 18		16	77	378	2 6 in 19 days
2306	25	S, A R	September 6		16	84	474	None
			March 10		16	78	370	1 2 in 4 days
2501	27	S, A R	September 14	77	17	81	432	1 3 in 2 days
			September 19	71	16	89	403	1 9 in 7 days
			September 27		16	102	369	3 4 in 15 days
			October 11	74	16	99	354	5 8 in 29 days
2539	46	S, A R, P T b	October 25	57	16	82	425	None
			October 28		16	82	375	0 9 in 3 days
			November 1		16	78	362	1 5 in 6 days
			November 6		18	93	314	3 2 in 12 days
			November 16		16	85	362	0 1 q d
2583	38	S, Aor- titis	December 17	54	16	111	443	None
			December 18		16	111	392	1 6 in 2 days

TABLE 6 (continued)

E.K.G. number	Age	Clinical diagnosis	Date	Weight	P R Interval	Heart rate	K +	Digitalis†
	years			kgm	seconds			grams
2618	54	S, A.R.	January 11	55	20	82	427	1 0 in 24 hours
			March 14	52	22	82	414	0 1 q d
			March 21		24	80	.394	1 0 in 7 days
2655	60	S, A.R.	February 11	65	16	81	413	None
			March 10		14	78	.365	1 0 in 4 days
			March 14	59	16	78	.361	1 7 in 7 days
			August 20	62	16	70	407	None for 1 month
2677	44	S, A.R., G.A.	February 28	79	16	98	416	0 8 in 6 hours
			March 1		17	100	403	1 4 in 3 days
			March 8	66	16	97	.386	2 1 in 10 days
			July 4		18	108	431	None for 4 weeks
			July 7		18	92	.347	1.5 in 3 days
			July 15	59	16	91	.363	0 1 q d
			August 4		20	113	.396	0 1 q d
			September 1		16	82	.333	0 1 q d
2709	40	S, A.R.	March 17	46	16	107	419	None
			March 18		16	87	391	1.2 in 24 hours
			March 20	44	17	83	.359	1 6 in 4 days
			March 23		17	72	390	None for 1 week
2776	41	S A.R.	April 29	62	15	95	451	None
			April 30		16	92	389	1 9 in 24 hours
			May 5	59	17	94	375	2 6 in 7 days
2819	50	S A.R., G A	May 6		16	82	421	None
			May 19		16	78	.370	1 0 in 3 days
2831	56	S, A.R.	May 29	71	16	90	428	None
			July 28		14	100	.388	0 1 q d
			August 1	65	16	91	.345	1 2 in 3 days
			August 20				402	None for 2 weeks
2855	44	S A.R. A F	June 17	80	17	69	.343	? Outside
			June 19		?	53	329	1 5 in 2 days
			June 24	78	16	83	.376	None for 5 days
2879	45	S A.R.	July 4	72	13	101	416	None
			July 14		16	88	.386	1.2 in 3 days
			July 21		16	86	.341	2 1 in 10 days
3017	46	S A.R.	November 7		15	70	450	None
			December 15	49	13	63	352	1 2 in 3 days
			June 16		13	56	424	None for 2 months
			June 19		16	57	.365	0 6 in 3 days

TABLE 6 (continued)

E.K.G. num- ber	Age	Clinical diagnosis	Date	Weight	P-R interval	Heart rate	K *	Digitalis †
	years			kgm	seconds			grams
3305	26	S, A R	June 22	49	16	99	437	None
			June 26		16	103	387	1 2 in 4 days
			July 3		16	98	403	2 0 in 12 days
3497	50	S, Aor- titis	October 22	56	16	56	410	None
			October 23		17	66	430	0 8 in 2 days
			November 17	49	18	86	412	4 6 in 27 days
C Hypertensive and arteriosclerotic heart disease								
1441	58	H, G A, S	August 9	49	17	88	378	1 8 in 8 days
			August 15	46	18	81	351	2 4 in 14 days
			October 11	52	17	86	468	None for 3 months
1649	28	H, Chr Neph	December 14 December 22	54	14 16	138 75	428 406	None 2 2 in 8 days
2010	36	H, G A, Em- physema	August 7	63	16	90	406	None
			August 14	59	16	87	388	1 8 in 7 days
			August 31	55	16	92	399	4 6 in 25 days
2221	61	H, G A	January 2	75	16	105	437	None
			February 25	70	16	95	397	2 1 in 21 days
			March 25		16	105	439	None for 10 days
			June 3	61	18	92	398	8 4 in 78 days
			March 10		16	100	451	None for 1+ month
			June 9		16	111	381	7 4 in 61 days
2422	59	H, G A, Cor Ob	March 18		16	120	424	None
			March 19	59	16	110	425	0 8 in 24 hours
			March 20		20	108	375	1 2 in 3 days
			March 25		20	100	369	2 0 in 7 days
			April 1	57	16	90	397	2 8 in 15 days
2440	51	H, G.A, Chr Neph	June 21	70	13	102	482	None
			June 22		13	100	408	1 3 in 24 hours
			June 24		14	98	404	1 5 in 3 days
			July 27	68	16	86	366	2 8 in 16 days
			August 19		15	93	428	None for 14 days
			August 23		16	83	424	1 1 in 5 days
			August 30		16	80	401	0 1 q d
			September 5		13	89	435	0 1 q d
			October 25		13	100	412	0 1 q d +1 0 in 5 days
			November 6		16	92	335	0 1 q d +1 6 in 6 days
			November 26	73	16	86	372	0 1 q d
2545	36	H, Chr Neph	November 27	66	16	66	458	None for 14 days
			December 24	63	16	70	387	0 9 in 24 hours
			January 3		16	64	378	1 8 in 10 days
			January 13		16	98	401	0 1 q d
			January 17		16	81	376	1 4 in 5 days

TABLE 6 (continued)

E.K.G. num- ber	Age	Clinical diagnosis	Date	Weight	P R Interval	Heart rate	K*†	Digitalis†
	years			kgm	seconds			grams
2638	48	H, G.A.	January 31		19	83	495	None
			February 3		20	86	456	0.8 in 3 days
			February 14	80	20	79	419	2.7 in 14 days
2648	53	H G.A. Chr Neph	February 10	70	16	95	460	None
			February 28		19	91	400	2.4 in 28 days
			March 11	65	18	59	388	0.1 q d
2694	70	G A, Em physema P T b	March 7	58	16	106	446	None
			March 8		18	87	.319	1.3 in 24 hours
			March 10		18	91	.308	1.7 in 3 days
			March 14	56	16	94	.356	2.2 in 6 days
2695	70	G.A., Hemi- plegia	March 7		16	61	421	None
			March 10		16	62	.396	1.0 in 4 days
			March 17		16	58	.391	1.7 in 11 days
2836	62	H Cor Ob Angina	May 30	54	28	65	.394	None
			June 9		.36	79	.367	1.6 in 11 days
2921	53	H G.A. Chr Neph, Bron- chial Pneu- monia	August 14		16	105	.523	None
			August 15		16	110	.358	1.2 in 20 hours
			August 16		16	110	.336	1.6 in 3 days
			August 18		16	118	.352	2.1 in 5 days
			August 23		20-40	70	?	2.7 in 10 days
3256	64	H, G.A. Aortic Dila- tation A F	October 17		16	77	.373	0.1 q.d
			October 19	60	?	72	.307	1.5 in 2 days
			October 20		?	68	.311	No more
			October 31		16	63	.327	0.8 in 8 days
			November 14		17	81	.406	2.2 in 22 days

\* "K" = "Q T" interval  $\sqrt{\text{"R R" interval}}$  Its average value for normal male Chinese is  $0.374 \pm 0.0012$  (1)

† See footnote to Table 1

duced the value of "K" from 0.431 to 0.347. In spite of the length of the interval in these cases, it would appear that this change is due to subliminal amounts of the drug remaining in the body. Case 2440 (Table 6) is instructive with regard to the maintenance of digitalis effect.

We have not extensively investigated the relation between the change in the relative length of systole produced by digitalis and the size of the heart. Cohn and Stewart (8) have shown that digitalis reduces the size of the heart in dogs and Stewart (9, 10) has extended the observa-

TABLE 7

*Effect of digitalis on the duration of the "Q-T" interval in 28 female Chinese with heart failure*

(Abbreviations as in Table 6)

E.K.G. number	Age	Clinical diagnosis	Date	Weight	P-R interval	Heart rate	'K' *	Digitalis †
	years			kgm	seconds			grams
A Rheumatic heart disease								
2095	35	M D	October 19		17	91	430	None
			October 26		20	65	342	2 2 in 7 days
			November 7		20	67	359	3 4 in 19 days
2160	12	M D (active) A F	December 3	29	20	92	378	0 6 in 3 days
			December 5		24	71	303	1 9 in 6 days
			December 7		?	53	343	2 1 in 8 days
			December 10		20	72	306	No more
2312	36	M D, A D	March 20		16	70	433	None
			March 25		15	74	356	1 3 in 5 days
2427	26	M D	June 10	43	20	82	420	None
			June 14		18	88	389	1 6 in 3 days
			November 6	42	20	78	429	None for 2 months
			November 11		20	63	382	1 2 in 4 days
2549	22	M D, A D	November 8		15	78	411	None
			November 11	53	17	73	398	1 2 in 4 days
2553	24	M D	November 13	45	20	100	455	None
			November 14		16	102	417	0 6 in 2 days
			November 16		16	98	412	1 0 in 3 days
			November 19		16	95	405	1 7 in 6 days
			November 21		17	108	376	2 0 in 8 days
2656	39	M D, Preg- nancy	February 12	59	14	95	401	None
			February 15		13	64	387	1 1 in 2 days
			February 18	57	15	57	354	1 7 in 6 days
2662	10	M D	Julv 4	34	16	120	465	None
			July 5		16	118	490	None
			July 7	33	16	111	463	None
			July 9		16	111	428	0 4 in 10 hours
			July 10		16	94	400	1 0 in 24 hours
			July 12		16	74	377	1 5 in 3 days
			July 15		16	93	409	0 1 q d
			July 21	27	20	86	370	0 1 q d
			October 20		16	110	490	None for 1 month
			October 25		24	100	405	1 5 in 5 days
2663	42	M D, A D	February 17	40	16	74	394	None
			March 7		16	81	354	1 2 in 4 days

TABLE 7 (continued)

E. A. G number	Age	Clinical diagnosis	Date	Weight	P R Interval	Heart rate	K' •	Digitals†
	years			kgm	seconds			grams
2734	26	M D, A D	April 3 April 8 May 16 May 19 May 27	52	17 20 18 16 20	97 94 97 97 105	411 375 417 355 344	None 1 6 in 6 days None for 14 days 1 0 in 4 days 1 4 in 11 days
2744	31	M D	May 22 June 19	52	16 20	94 61	411 388	None for 2 months 4 7 in 30 days
2799	22	M D	May 8 May 12 May 23	38  34	15 12 20	100 98 51	411 320 384	None 1 8 in 5 days 3.2 in 16 days
2918	24	M D	August 13 August 14 August 16 August 21 August 26 September 16	53   44  	28 .32 .32 .32 .32 28	87 74 73 75 70 76	432 439 420 396 374 353	None 1 1 in 2 days 1.3 in 4 days 2 0 in 10 days 2 6 in 14 days 5 8 in 34 days
2931	37	M D	August 30 October 13 November 30 December 1	40	16 12 16 13	84 103 106 90	423 394 381 367	None 4 0 in 44 days 0 1 q d 0 1 q d
3015	25	M D, Preg nancy A F	March 7 March 14 February 2 March 4 March 13 May 25	  46   	18 20 25 ? 20 20	102 120 88 165 106 87	416 396 314 331 375 433	None 1.8 in 7 days 2.3 in 8 days 1 2 in 2 days No more No more
3232	28	M D  A F	April 28 April 29 May 1 May 4	46   41	18 24 ? 20	103 86 78 71	380 304 273 333	? Outside 1 2 in 24 hours 0 1 q d 0 1 q d
3280	20	M D A D (active)	June 8 June 9 June 10 June 11	50	17 20 20-.39 .32	98 71 63 66	410 343 317 294	None 1 2 in 24 hours 1.5 in 2 days 1 6 in 3 days
3349	9	Acute Car ditis	August 1 August 3 August 5	24	16 16 20	130 107 118	361 334 322	None 0 6 in 2 days 0 9 in 5 days
3354	30	M D	August 6 August 18	42	16 16	85 60	415 345	None 1 5 in 6 days
3478	21	M D, A D (active)	October 15 October 28 October 29 October 30 November 9	  38  	16 40 24 20 22	79 50 39 56 54	366 298 313 345 378	? Outside 0 8 in 24 hours No more No more 1.2 in 14 days

TABLE 7 (continued)

E.K.G num ber	Age	Clinical diagnosis	Date	Weight	P-R interval	Heart rate	K *	Digitalis†
	years			kgm	seconds			grams
<i>B Syphilitic heart disease</i>								
3364	39	S, A R	August 13	49	12	55	405	0.6 in 24 hours
			August 20		13	49	396	1.2 in 7 days
<i>C Hypertensive and arteriosclerotic heart disease</i>								
1777	51	H, G A	February 2		15	91	448	None
			February 8		16	80	430	1.6 in 7 days
1785	39	H, S	February 14	60	20	86	424	None
			March 10		20	68	348	3.8 in 25 days
1890	48	H, G A	December 5		15	75	407	None
			December 7		15	60	402	1.2 in 2 days
1983	23	H, Ac. Neph	July 19		12	120	401	None
			July 23	51	15	94	316	1.8 in 4 days
2343	43	H, G A	March 30	52	16	82	440	None
			April 15		16	68	417	1.1 in 3 days
2643	43	H, G A, Chr Neph, S	March 15	40	16	95	477	None
			March 17		16	82	415	1.2 in 2 days
			March 24	34	18	73	398	2.6 in 9 days
			April 1		18	60	362	4.1 in 17 days
			April 7	30	20	61	382	0.1 q d
2666	37	H, Chr Neph	February 19	54	16	109	484	None
			February 28		16	105	409	1.2 in 24 hours
			March 3		16	104	384	1.7 in 5 days
			March 8	48	16	86	365	2.3 in 10 days
			March 17	44	17	93	346	4.0 in 20 days

\* "K" = "Q-T" interval  $\sqrt{\text{"R-R" interval}}$  Its average value for normal female Chinese is  $0.388 \pm 0.0015$  (1)

† See footnote to Table 1

tion to normal persons and to patients with heart failure. In general our data (not presented here) agree with these results. Under various circumstances there are exceptions and it must be noted that in some of these the relative duration of systole is decreased, although the heart size remains the same or is increased, in a few instances the reverse combination occurs (see Table 8). These exceptional cases are for the most part among patients with an actively progressive infection of the heart. There has so far not been demonstrated any constant relation between heart size, aside from heart failure, and relative length of systole, but this question is of such importance as to demand further careful study.

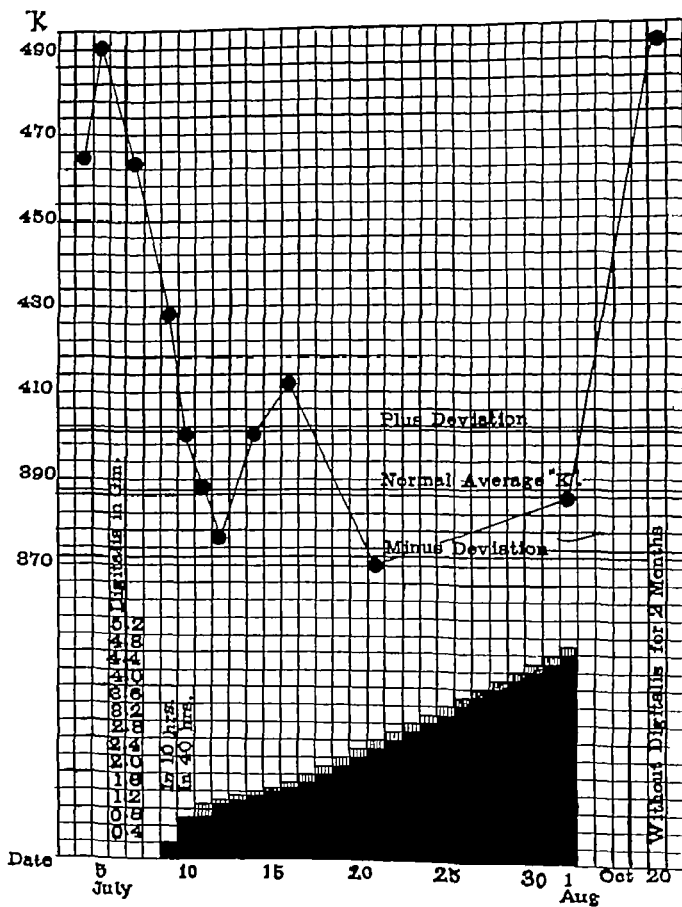


FIG 1 E K G NUMBER 2662, TABLES 1 AND 7

After 1.3 gram of digitalis the value of 'K' (see text) rapidly falls to within normal limits. With 0.1 gram a day the value rises above normal (July 16) but with 0.2 gram a day it remains normal. After two months without digitalis 'K' is far above normal.



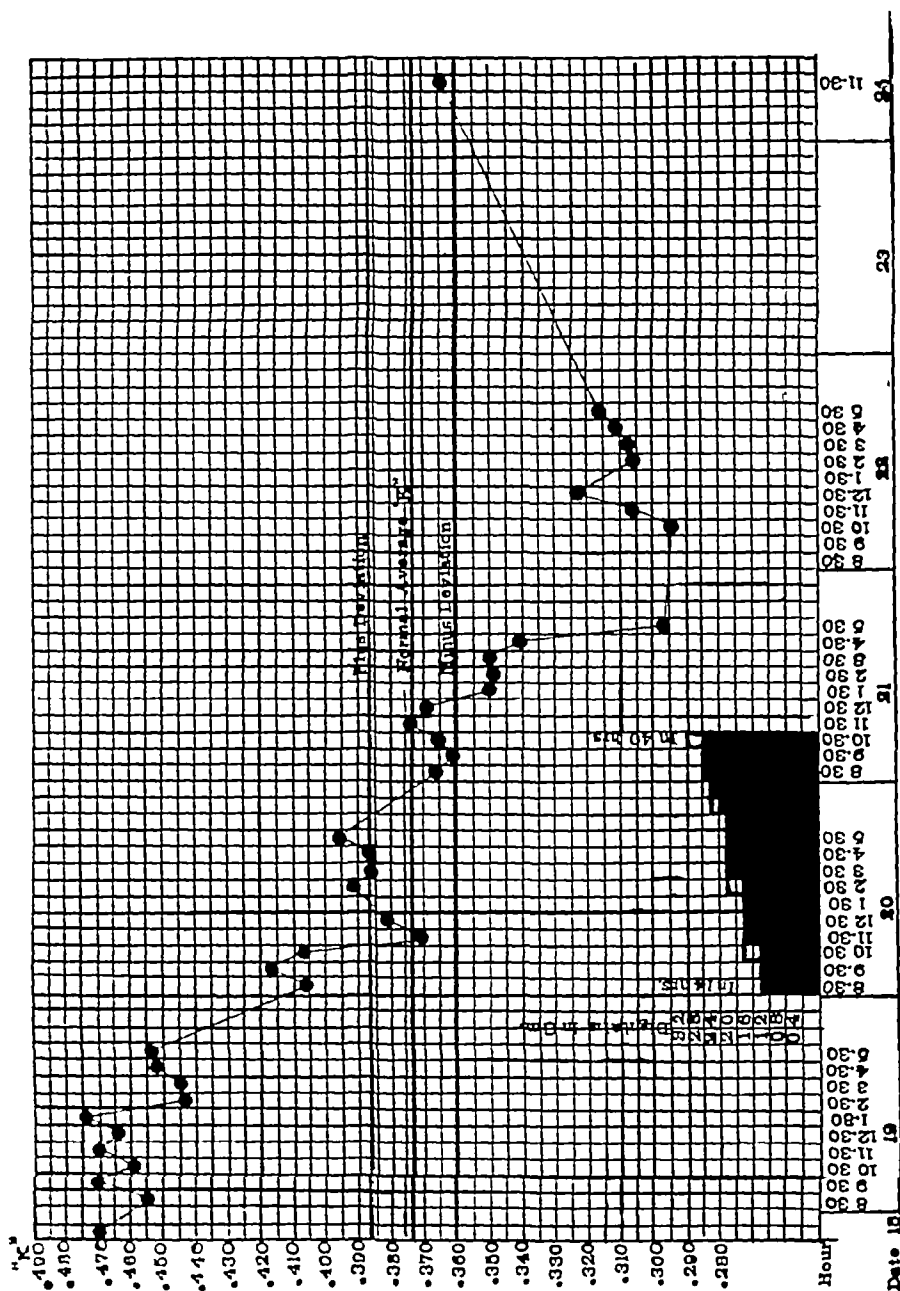


FIG 2 E K G NUMBER 3053, TABLE 2

The value of "K" (see text) is rapidly reduced to within normal limits and after a large dose of digitalis (3.2 grams) falls below normal

TABLE 8

*Changes in heart size and relative length of systole during digitalis therapy*

E.h.G number	Sex and age	Clinical diagnosis	Date	Weight	Heart failure*	Heart rate	K †	Digitalis†	Heart over size
	years			kgm				grams	sq cm
1886	M 19	M D (active)	May 15	47.5	I	88	406	None	+6
			June 4	41.7	0	75	348	3.3 in 17 days	+50
			June 18	41.7	0	70	350	3.8 in 24 days	+16
3053	M 21	M D A D	November 18	44	III	102	470	None	+51
			November 21	42	IIa	94	260	3.2 in 3 days	+47
3100	M 27	M D	May 13	85.2	IIb	97	405	None	+53
			May 19	56.6	I	80	368	3.0 in 4 days	+37
			May 27	52	I	79	367	0.1 qd	+36
			June 2	56	I	93	372	0.1 qd	+42
			October 16	55	I	92	432	None for 3 months	+25
2662	F 11	M D A D (active)	July 18	26	IIa	93	409	1.8 in 8 days	+44
			October 24	26	I	100	405	1.5 in 4 days	+22
			January 16	28	IIb	120	460	None	+63
			March 2	26	I	115	443	39 days after 1.9 in 19 hours	+35
			June 8	31	I	99	463	None for 2 months	+25
			November 26	35	I	103	428	None for 2 months	+30

\* Classified according to the criteria of the New York Tuberculosis and Health Association

† See footnotes to Tables 1 and 2

## DISCUSSION

It is still impossible to measure satisfactorily the work done by the human heart. One factor which must enter into a consideration of this problem is the duration of systole. We have shown that this is increased in heart failure out of proportion to the rise in heart rate. Heart rate is another factor of importance and in failure is usually elevated to some degree.

The results of the heart's work are shown in the blood pressure, which, except in the case of auricular fibrillation, is usually well maintained, and in the cardiac output per minute, which is usually decreased in failure. In spite of the fall in mass movement of blood, it would seem as if the work of the heart was not decreased, but rather is inefficiently performed. Calculation of the time occupied by systole in our cases shows that it may be increased to twice the average normal length. The known changes in the direction of increased efficiency brought about by digitalis are slowing of the heart and second relatively great increase in systole. Not infrequently the second result may be of

With regard to the mass movement of blood, the work of Cohn and Stewart shows that in recovery from heart failure the significant change is toward more efficient emptying of the ventricles, for in spite of decreases in heart size and rate, the cardiac output per minute increases. As we have already suggested one would expect in this connection some relation between heart size and contraction time.

There are many reasons for believing that the effect of digitalis under discussion is chiefly exerted directly upon the myocardium. Vascular changes cannot be excluded, but would seem to be secondary. In congestive failure there is always an increase of venous pressure (11), which is apparently a reflection of the decreased mass movement of blood. The fall in venous pressure which accompanies improvement in the circulation (12) must go hand in hand with decreased diastolic volume of the heart and may be related to the shortening of systolic time.

It is desirable to emphasize the fact that the various aspects of the efficiency of the circulation cannot be considered separately, but are intimately interrelated. There is always danger of serious error in starting with one factor and arguing that various changes "result" from its operation. Nor should we be too quick to apply the normal laws of physiology to pathological conditions. In spite of the importance of ventricular filling under normal conditions, it does not seem that this factor operates toward the decreased cardiac output per minute in heart failure, for the ventricles are apparently filled to an abnormal extent.

The duration of systole in relation to cycle length would appear to be a valuable guide to digitalis therapy. Reference to a chart such as that presented in our previous article (2), immediately shows the relation of the values obtained to the normal limits. The changes are much more delicate than those in the "*P-R*" interval and often much clearer than those in the "*T*" wave. Our experience has led us to believe that excessive use of digitalis is no more desirable than insufficient use and the relative length of systole has proved a delicate guide to the danger of overdosage.

#### SUMMARY

An electrocardiographic study was made of the action of digitalis on the "*R-R*" and "*Q-T*" intervals of patients with heart failure. A consistent decrease was found in the length of the "*Q-T*" interval in relation to the "*R-R*" interval, which was often decreased. This reduction was not always paralleled by a decrease in heart size. It is apparently an important index of the greater efficiency of the myocardium in recovery from heart failure, and is interpreted as the result of a direct action of digitalis on the myocardium. The relative length of systole is a good guide to digitalis therapy.

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## STUDIES OF SERUM ELECTROLYTES

### VIII THE CONCENTRATION OF ELECTROLYTES AND NON ELECTROLYTES IN THE SERUM FOLLOWING INSULIN ADMINISTRATION IN DIABETIC PATIENTS

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Many studies have appeared in the literature on the effect of insulin on various constituents of the blood or blood serum of normal and diabetic individuals These have been reviewed by Peters and Van Slyke (1) The evidence in the literature indicates that coincident with the fall of glucose, insulin produces a simultaneous fall in the concentration of inorganic phosphate, potassium and cholesterol in the serum The amino acids appear to be lowered, lactic acid and nonprotein nitrogen apparently are not significantly altered, and the blood calcium concentration may be increased There seems to be a difference of opinion as to the effect of insulin on the blood volume and the water content

Gram (2) noted that the osmotic pressure of serum from diabetic patients was above the normal range Hartmann and Darrow (3) observed that in two of their cases of diabetic acidosis in children who were treated with insulin, water, and carbohydrate, the observed osmotic pressure decreased as much as eighteen per cent following therapy The freezing point of serum taken from control rabbits Haldane, Kay and Smith (4) found to be the same as from litter-mate rabbits after insulin convulsions, from which they conclude that insulin does not alter the osmotic pressure of the serum of normal rabbits

Whether these changes described are caused by insulin alone or by other features of the total therapy, it is frequently difficult to determine The effect of insulin on the blood serum may possibly be different in normal and in diabetic individuals, with and without ketosis, as well as in different animal species

The following studies were planned to give a picture of the correlated changes occurring in the various electrolyte and non-electrolyte components of the blood serum of fasting diabetic patients with hyperglycemia but without ketosis, after a large dosage of insulin

## MATERIAL AND METHODS

Seventeen observations have been made on patients suffering with severe diabetes mellitus who were attending the Metabolic Clinic at the Pennsylvania Hospital. After a period without insulin (generally twenty-four to forty-eight hours) long enough to induce a marked elevation of the blood sugar, and after an overnight fast, sufficient blood was taken by vein for the complete analyses. The patients, still fasting, were then given from 50 to 150 units of insulin, usually at one injection. In several instances, however, when no significant decrease in the value of the capillary blood sugar had occurred within one hour after the first injection of insulin, a second dose was administered. The total dosage is recorded in Table 1. Following insulin, the patients were permitted water as desired. At intervals of twenty minutes, finger-prick blood sugar values were obtained with a technique by which analytical results could be secured within fifteen to eighteen minutes after the withdrawal of the blood. As soon as the concentration of blood sugar had fallen to within normal limits (forty minutes to three hours) or upon the appearance of any symptoms of an insulin reaction, a second specimen of blood was removed by vein for complete analyses and the patient was returned to his usual regimen.

The analyses included serum chloride, carbon dioxide content, inorganic phosphate, total base, sodium, conductivity, freezing point, refractometric index, specific gravity, dry weight, cholesterol, nonprotein nitrogen, and blood sugar.

The chemical methods used in previous studies of this series were employed. Freezing point measurements were made, using refinements described recently by Sunderman (5). Conductivity measurements were made with a special conductivity assembly (6). For estimation of the protein of the serum from specific gravity, the equation of Moore and Van Slyke was employed (7). The Folin and Malmros sugar method (8) was used. The pH of the serum was measured in a single case with the glass electrode by the method of Stadie, O'Brien, and Laug (9).

## RESULTS

The values of the individual analyses before and after insulin are recorded either in Figure 1 or in Table 1. In Figure 1 the dots represent original values before insulin, and the arrow points, values after insulin. In Table 1 are included dry weights and in many instances specific gravity of the sera. These data permit the calculation of individual components in relation to either dry residue or water. In our discussion of the data, however, the values are expressed in relation to total serum volume.

*Blood sugar.* The sugar values given in Figure 1 represent analyses made on whole venous blood without anticoagulant, the proteins having been precipitated by tungstic acid immediately after withdrawal of the

TABLE 1  
Individual case analyses

Case number	Insulin dose	Time after insulin	Serum total base	Serum CO <sub>2</sub>	Serum nonprotein nitrogen	Serum specific conductivity (K)	Corrected conductivity	Serum specific gravity		Serum dry weight	Refractive index calculated as serum protein	Serum pH
								Observed	Calculated as protein			
	units	minutes	mEq per liter	mM per liter	mgm per 100 cc.	mhos $\times 10^{-4}$ at 85	mEq NaCl per L.	$\frac{80}{80^\circ}$	grams per cent	grams per liter	grams per cent	
1	120	B* 125	138 141	25.4 26.5	30 31					96.0 99.4		
2	50	B 40	148 149	30.7 30.4	26 26	117.4 120.9	135.2 138.4			92.3 87.5	8.2 7.8	
3	150	B 370	141 143	28.1 32.9	27 24	108.6 117.4	119.9 133.0	1.0284	7.4	94.1	8.5 7.7	
4	50	B 170	148 151	27.4 29.2		119.2 121.0	136.5 140.5	1.0285 1.0284	7.4 7.4	90.0 91.5	7.9 8.3	
5	75	B 175	141	29.2 28.7		117.4 120.1	133.9 141.4	1.0269 1.0282	6.8 7.3	84.1	7.8 8.9	
6	75	B 180	144 148	23.8 27.7		113.1 115.7	130.4 136.0	1.0289 1.0292	7.5 7.5	91.3 96.2	8.3 8.9	
7	50	B 105	146 151	26.3 28.7		117.4 119.2	134.9 137.5	1.0256 1.0283	6.4 7.3	91.2 92.0	8.1 8.2	
8	50	B 105	145 150	26.4 26.1		120.1 121.9	138.6 140.8	1.0254 1.0260	6.3 6.5	91.1 91.8	8.2 8.1	

\* B—before insulin





blood The average diminution in the concentration of sugar was 178 mgm per 100 cc in 145 minutes With four of the patients (Cases 9, 10, 11, 17) the study had to be terminated at a time when the concentration of sugar was above 200 mgm per 100 cc because of the appearance of shock like reactions In Cases 10, 11, and 17 the ingestion of orange juice and in Case 9, the intravenous administration of glucose quickly relieved all symptoms of these reactions

*Cholesterol* All of the cholesterol concentrations of the serum obtained from the patients before the injection of insulin were above the normal range and varied from 196 to 322 mgm per 100 cc (Figure 1) Coincident with the fall in sugar the concentration of cholesterol consistently decreased

*Nonprotein nitrogen* values (Table 1) were scattered about the normal range except in Case 17 in whom, both before and after insulin, this component was slightly above normal

*Solids* The concentration of total dry substances as shown in Table 1 exhibited no consistent change although there was a tendency for the specific gravity to increase In a series of measurements on medical students, Dr E Harper working in our laboratory found the protein of the serum by means of specific gravity to be from 7.6 to 8.5 grams per cent with an average concentration of 7.84 grams per cent The protein, calculated from the specific gravity in our series of observations was consistently below this normal range both before and after insulin

*Anions* It will be observed in Figure 1 that the *inorganic phosphate* of the serum decreased in each observation, as was anticipated from the studies of others This simultaneous fall in the concentration of inorganic phosphate and sugar has led a number of investigators to suggest that insulin may be concerned in the formation of hexose-phosphoric esters in the tissues

In each of our observations the *chloride* concentration in the serum increased after insulin administration, the percentile increase amounting to as much as nine per cent It will be seen that in the original sera the chloride concentration was below the normal range in 13 out of 17 measurements The chloride content of sera obtained after insulin increased either to or toward the normal range and in one observation above it In 12 of 17 analyses the *carbon dioxide* content rose.

*Cations* The measurements of the concentrations of cations in the sera were limited to *total base* and *sodium* The results of the individual analyses of total base are given in Table 1 and of sodium in Figure 1 It will be seen that in 8 out of 13 observations the concentration of sodium in the serum before insulin administration was below the normal range In each case after insulin the concentrations of sodium increased above the original values and in 5 analyses, above the normal range The changes of total base were similar in direction and magnitude to

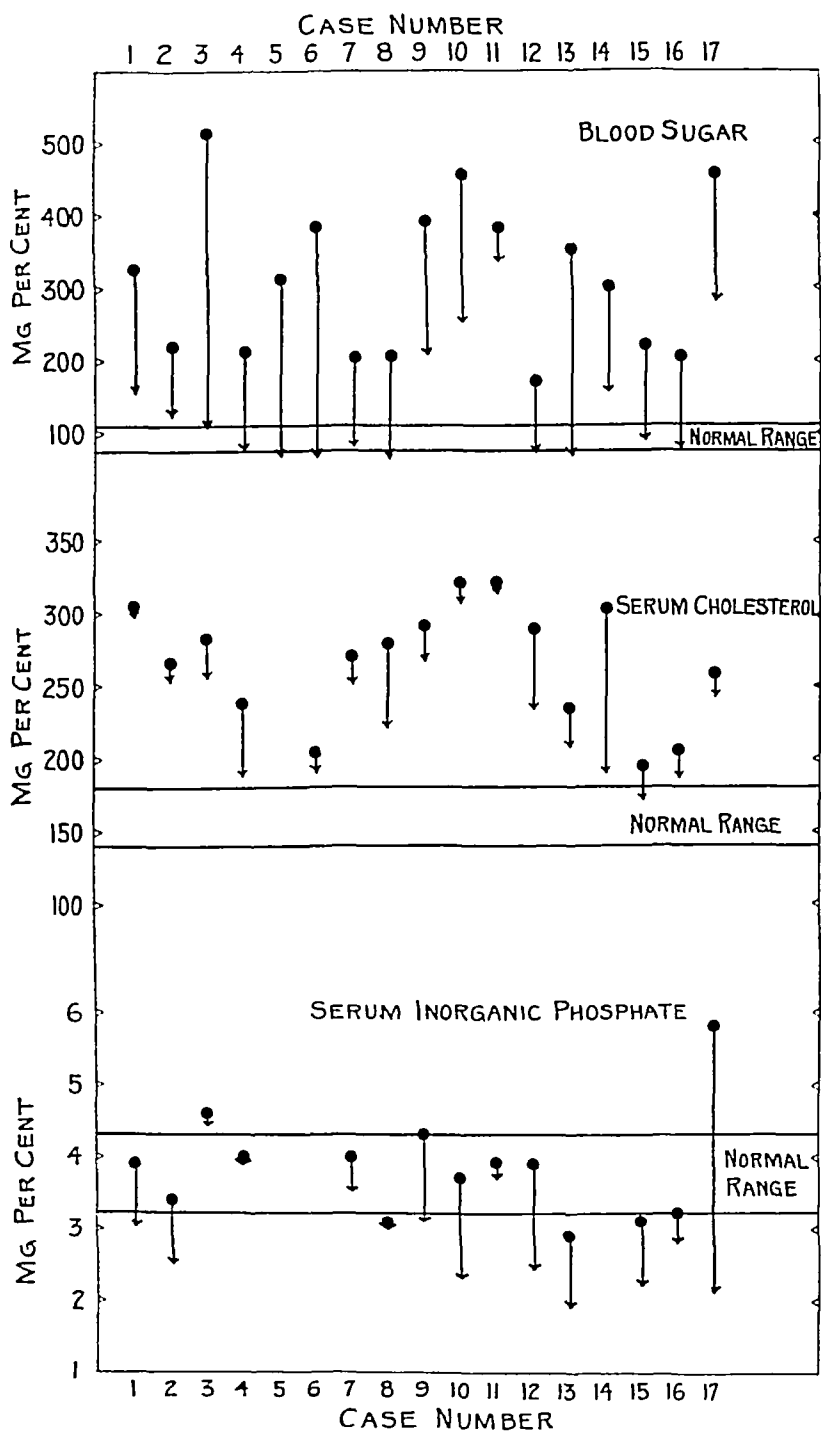


FIG 11

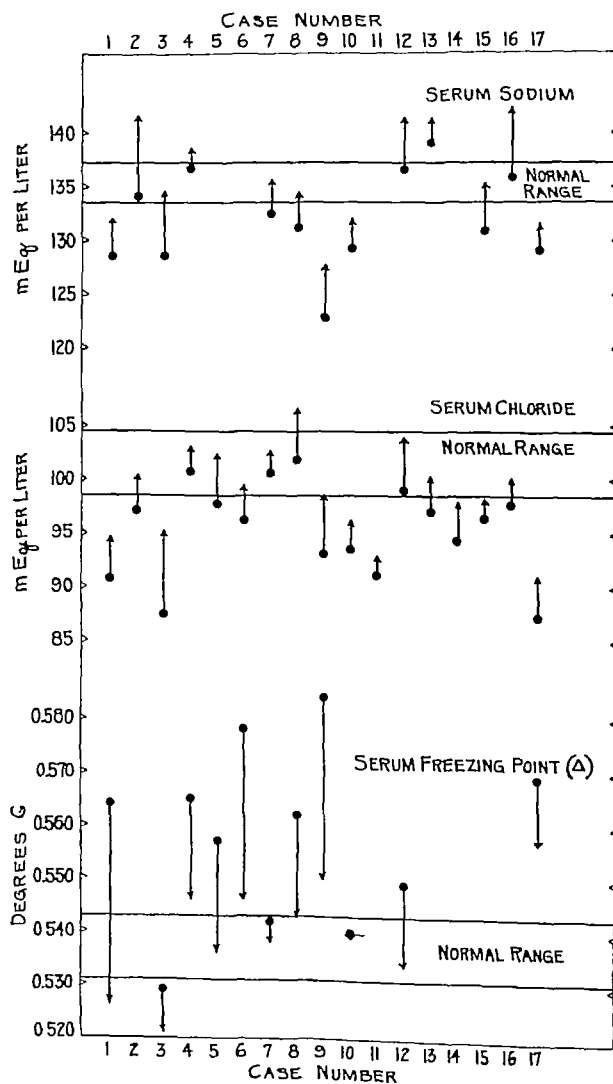


FIG 1B

those of sodium. This was to be expected since the sodium in serum represents from 90 to 95 per cent of the total base.

The increase in electrolyte concentration was also reflected in increased conductivity values of the serum following insulin. The conductivity values given in Table 1 are expressed both in terms of specific conductivity and in m Eq of NaCl, corrected for protein by the Gram and Cullen formula (10).

*Freezing point.* The values for the depression of the freezing point of serum before and after insulin therapy are given in Figure 1. In all but one of the observations the freezing point depression was less after insulin, the maximum difference being as much as  $0.034^{\circ}\text{C}$ . The freezing point depressions of the sera before insulin were generally greater than the normal range.

TABLE 2

*The freezing point of serum of normal fasting individuals*

Specimen	$\Delta$ $^{\circ}\text{C}$
FW S	0.533
Pe	0.538
PW	0.531
BH	0.532
FS D	0.543
Average	<u>0.5354</u>

In Table 2 are given values for the freezing point of serum obtained from normal fasting individuals, using the refinements (5) of the Stadie-Sunderman method (11). These values vary from  $0.532^{\circ}$  to  $0.543^{\circ}$  and are less than those recorded in the literature by observers who have used the Beckman method. Because of the supercooling and the increase of concentration produced by freezing out of solvent, the Beckman method tends to give unduly great depressions.

#### DISCUSSION

The reciprocal change of glucose and electrolytes observed in the blood serum appears to be similar to the inverse changes described by Herrick (12) following a sugar tolerance test and by Ni (13) following extirpation of the pancreas in a dog. In Ni's experiments, after pancreatectomy in the dog there was a reduction of the chloride of serum associated with an increased concentration of sugar. When insulin was given, sugar decreased and chloride rose. He estimated that only about 50 per cent of the osmotic pressure change produced by the blood sugar rise after pancreatectomy was compensated for by the decrease in chloride. From estimations of the osmolar changes owing to the decrease in glucose and the increase of electrolytes in our observations it would appear that the increase of electrolytes only partly compensated

the loss of glucose As direct evidence of this there was, as a rule, some fall in the osmolar concentration, as measured directly by the freezing point The studies in the literature and our observations would suggest that when the blood sugar falls or rises some tissue or body fluid, concerning the identity of which we have no evidence, is capable of coincidentally releasing electrolyte into or withdrawing electrolyte from the serum

The decreased concentration of cholesterol in the serum following insulin is consistent with the evidence in the literature (Bliv (14)), (Christomanos (15)) of the reduction not only of cholesterol but of lipoids in general While Nitzescu, Popescu-Inotesti, and Cadariu (16) found the hyperlipemia of both experimental and clinical diabetes to be reduced by insulin, no effect on the cholesterol content was observed in normal individuals We have made no measurements of the effect of insulin on the serum of normal subjects Rabinowitch (17) reported two hyperlipemic diabetic subjects in whom a rapid fall in the blood fat occurred after insulin therapy

In the four observations in which shock like reactions appeared at hyperglycemic levels, the question might be raised as to whether the sugar in arterial blood was also elevated at the time of the reactions We have no analyses of the sugar of arterial blood in these patients, but the values for capillary blood sugar which were obtained at intervals of twenty minutes in each experiment were always above the final venous blood sugar values

Shock-like reactions at high sugar values occurred in our patients, in four out of six instances, when high initial sugar values (above 380 mgm per 100 cc) were associated with rapid fall (1 mgm per minute or greater) It seems that neither high initial blood sugar nor rapid rate of fall will independently induce reactions at high sugar levels but that the combination of these two factors may do so It is also evident that reactions which we did not distinguish from ordinary insulin reactions and which were promptly relieved by the administration of carbohydrate can develop while the blood sugar concentration is above 200 mgm per cent under the conditions of these experiments As Joslin (18) points out this combination of events is extremely rare after insulin administration in ordinary clinical practice

#### SUMMARY

Insulin was withheld for 18 or more hours from 17 patients with severe diabetes mellitus After an overnight fast blood was removed for the analyses and the patients were given approximately a day's maintenance dosage of insulin, usually at one injection Food was withheld and capillary blood sugar measurements were made at intervals of twenty minutes until the concentration of blood sugar reached a normal level or

until the patients experienced early symptoms of an insulin reaction. At this time blood was again removed for analyses.

The concentration of inorganic phosphate and cholesterol of the serum after the insulin administration decreased, as had been anticipated. The concentration of total base, sodium, chloride, and specific conductivity of the serum following the insulin injection increased. As measured by freezing point, the osmolar concentration of the serum following insulin administration generally decreased, suggesting that the increased concentration of electrolytes did not compensate completely for the decreased concentration of the blood sugar. Specific gravity tended to increase above the original value after the administration of insulin. The final picture, we believe, represents a change in the serum toward the normal, not only in the fall of glucose and cholesterol, but also in the rise of sodium and chloride and the diminished osmolar concentration, as measured by the freezing point.

Shock-like reactions in four of our patients occurred at a time when the concentration of the blood sugar was above 200 mgm per cent. These reactions, therefore, do not seem to be necessarily caused by a hypoglycemic state. They occurred when the blood sugar fell rapidly from an initially very high level.

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## STUDIES OF CALCIUM AND PHOSPHORUS METABOLISM XX THE HIGH CALCIUM EXCRETION IN EXOPHTHALMIC GOITER IS NOT DUE TO VITAMINE D DEFICIENCY

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In previous publications of this series (1, 2), it has been demonstrated that exophthalmic goiter is associated with a calcium and phosphorus excretion elevated in excess of the increased general metabolism. Several conditions have a high urinary calcium excretion. As far as is now known, such a high calcium output when elevated in both urine and feces is found only in hyperthyroidism and vitamine D deficiencies (3). A patient with osteomalacia, carefully studied by Gargill, Gilligan, and Blumgart (4), showed an analogous high urinary and fecal calcium excretion, which returned to normal after large doses of vitamine D. It was, therefore, of interest to determine whether hyperthyroid cases, because of the elevated metabolic demand, could be suffering from vitamine D deficiencies due to an increased need for vitamins. The idea gains some support in the work of Plimmer (5) of Cowgill and Klotz (6) and of Himwich, Goldfarb and Cowgill (7), which indicates that the amount of vitamine B required by the organism is determined chiefly by its caloric requirement. In hyperthyroidism the publications of McCarrison (8) have directed attention to the relation of diet and hyperthyroidism, and Rabinowitch (9) and Fraser and Cameron (10) have published observations which suggested that the addition of vitamins A and D to iodine gave a somewhat greater improvement in Graves' disease, than did iodine alone. In the following observations, this problem was studied by giving irradiated ergosterol to hyperthyroid patients who were maintained on an otherwise constant regime.

### METHODS

The same careful metabolic routine fully described in previous publications (11), was followed. Further descriptions of this technic need not be added here. The diet was essentially neutral in its acid-base contents as calculated from Sherman's tables (12). The constituents were similar to those of Table VII in paper XI of this series (13), though the total amounts were increased to conform with the caloric needs of

exophthalmic goiter patients. Bacon, because of its varying content of sodium chloride was replaced by egg white and lactose. The calcium, phosphorus, and nitrogen contents of this diet were checked by actual analyses. The analytical methods which we used were Titratable acidity, modified after Henderson and Palmer (14), ammonia by the permutit-nesslerization method of Folin (15), calcium and phosphorus by Fiske's methods (16), and nitrogen by the Kjeldahl method.

#### DATA

The two patients were carefully selected as classical examples of exophthalmic goiter. One of them (AL) had the deformity of an old bone tuberculosis with Pott's disease, but apparently this had been inactive for many years. The patients were most cooperative and regularly ate their entire diet, so that they remained on a very constant regime. The actual periods of the observations were not begun until after RC had rested in bed without medication for six days and AL for ten days. As a result, the basal metabolic rate remained remarkably constant throughout the study until the addition of Lugol's solution to their regime caused its usual effect. Such a steady state was obviously essential in observations such as are here recorded. Because the results were so obvious and conclusive, only two observations have been made. The collected data are shown in Table I. They again demonstrate the high calcium and phosphorus excretion which occurs in exophthalmic goiter. This high excretion was maintained, for at least four weeks, in spite of a neutral diet, a positive nitrogen balance, and complete rest for the patient. The average three-day excretion of normal individuals on a neutral diet with similar intake was found to be 572 mgm., of which 386 mgm. were fecal. In these small women the calcium excretion was, therefore, four times the normal.

The addition of large amounts of irradiated ergosterol did not diminish the urinary calcium excretion nor influence the unusually high fecal excretion of calcium. This was true even though enough was given to slightly elevate the blood calcium levels. The response here is very different from that seen in Blumgart's case (4) and in the study of Bauer and Marble (17) who showed that vitamin D reduced the fecal calcium excretion dramatically in a patient suffering from steatorrhea.

The addition of irradiated ergosterol to the diet likewise caused no significant change in the basal metabolic rate, or in the urinary excretion of ammonia or titratable acids.

In both patients, however, there was a more marked nitrogen retention associated with its ingestion.

It is, therefore, clear that the high calcium excretion found in hyperthyroidism is not due to a deficiency of vitamin D.

TABLE I

Three-day periods

Pa- tient	Period	Medication	Basal meta- bolic rate	Weight	Titratable acidity —CO <sub>2</sub> in urine	Am- monia in urine	Titratable acidity plus am- monia	Calcium				Phosphorus				Nitrogen			Serum values	
								Urine	Feces	In- take	Balance	Urine	Feces	In- take	Balance	Urine	In- take	Balance	Cal- cium	Phos- phorus
			per cent	lbs	cc. N/10	cc. N/10	cc. N/10	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	mgm per 100 cc	mgm per 100 cc
AL	I	Control low cal- cium diet	+65	94.4	811	778	1589	192	100	.33	-2.59	290	88	2 20	-1.58	39.7	44.3	0.2		
	II		+65	92.0	812	906	1718	212	83	.33	-2.62	271	90	2 25	-1.36	41.7	47.1	0.7	10.4	5.2
	III	Same diet plus 1½ cc. viosterol per day (Squibb's 250 D)				691	1263	180	147	.33	-2.94	223	1.51	2 25	-1.49	33.3	47.0	9.0		
	IV	Same diet plus 3 cc. viosterol (Mead 250 D) per day	+54	92.8	572	804	1568	193	13	.33	-1.73	262	.22	2.25	-0.59	34.9	47.0	7.4	12.2	5.5
	V	Same diet plus 3 cc. viosterol (Mead) per day plus 15 tablets Osodal* per day	+69	89.3	910	946	1856	182	174	.33	-3.23	282	1.30	2 24	-1.88	34.2	45.9	7.1	11.6	6.2
	VI	Control	+62	89.0	888	753	1641	197	86	.33	-2.50	286	84	2 24	-1.46	33.6	47.0	8.7	11.6	5.7
	VII	Same diet plus Lugol's, 10 drops t.i.d.				809	1556	218	105	.33	-2.90	271	93	2 24	-1.40	38.2	47.1	4.2	12.8	6.6
	VIII	"	+57	89.8	639	731	1370	177	126	.33	-2.70	242	1.33	2 24	-1.51	32.5	47.1	9.9	11.6	5.3
	IX	"	+40	90.0	402	790	1192	146	62	.33	-1.75	197	1.00	2 24	-0.73	31.1	47.1	11.3	10.6	4.4

remarkable X-rays of the chest disclosed nothing abnormal Her temperature was normal throughout the observation

After the studies reported in this communication, she returned to the Massachusetts General Hospital, where an uneventful subtotal thyroidectomy was performed Two basal metabolic rates determined there showed the postoperative rate to be minus 21 per cent and minus 25 per cent.

A pathological report of the thyroid, made by Dr J I Bradley at the Massachusetts General Hospital, was as follows "The specimen weighed 110 grams Microscopic examination showed numerous small and medium sized acini lined by high cuboidal epithelium and filled with a moderate amount of vacuolated colloid The majority of the acini showed rather marked papillary proliferation of the epithelium In some areas the papillary projections are so marked that the lumina of the acini are almost occluded There is a slight fibrosis and a few focal lymphocytic accumulations are present

"Hyperplasia, exophthalmic type"

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eral factors may be of great importance and that the circulatory disturbances of emphysema may be due to obstruction in the return flow of venous blood. At least they suggest that the effect of intrapleural pressure should be studied before conclusions are drawn concerning the site of the circulatory block.

The only direct observations on the rate of circulation in emphysematous patients were made by Blumgart and Weiss (5). They injected a radioactive substance in the vein of one arm and determined the time required to detect the emanations in the other arm. They found no striking variation from the normal in patients with moderate emphysema but did find a moderate increase in the circulation time in advanced cases of the disease.

The following investigations were designed to offer information concerning the state of the circulation in emphysema and the influence of the altered pressure relations in the chest. The problem was attacked directly in dogs in which an artificially produced emphysematous state existed and in patients as far as experimental methods could be applied.

Intrapleural and venous pressures (White method (6)) were determined in normal subjects and in the same individuals during the performance of a modified Valsalva experiment. Similar determinations were made on emphysematous patients with and without exacerbations of moderate respiratory distress. The circulation rate was determined in normal subjects and in emphysematous patients.

The technic for measuring the intrapleural pressure in patients consisted of having the subject lie on his left side with his right arm above the head and the hips slightly elevated. The chest wall was leveled using a spirit level as a guide. Under novocaine anesthesia a number 16 thoracentesis needle was inserted into the chest and connected to a water manometer. About 35 cc of air were injected into the pleural cavity to keep the lung away from the needle. This quantity caused no change in the manometer reading. As the volume of the respiration influences the intrapleural pressure, it was necessary to have all subjects breathe without force into a tank which measured the expired air. The reading of the intrapleural pressure was also taken in normal subjects when the tidal air became constant at 500 cc. Three normal patients were caused to take a deep breath and cautiously make expiratory effort against the closed glottis (Valsalva experiment). The same technic could be followed in moderately dyspneic patients with emphysema but individuals who were extremely dyspneic could not be studied due to the discomfort of the position. In no cases were full expiratory efforts exerted by the individual.

The intrapleural pressure of patients who did not have emphysema was found to average  $-4$  to  $-8$  cm of water at the time that the tidal air was 500 cc or less. The peripheral venous pressure of these individuals

TABLE I

*Intrapleural and venous pressures in normal individuals and emphysematous patients*

Patient	Vital capacity	Tidal air	Intrapleural pressure* tidal air		Intrapleural pressure. Tidal air 500 cc.		Intrapleural pressure. Val-salva experiment	Venous pressure	Venous pressure during asthmatic attack	Venous pressure at end of Val-salva experiment
			Inspiration	Expiration	Inspiration	Expiration				
	cc.	cc.	cm. water	cm. water	cm. water	cm. water	cm. water	cm. water	cm. water	cm. water
1 Normal	3800	375	-4	-6	-5	-7	+6	4.5		18
2 Normal	4200	425	-3	-7	-3	-9	+8	5		16
3 Normal	4500	480	-4	-8	-4	-8	+10	5		18
4 Emphysema	4100	380	-2	-5			+12	6	10	14
5 Emphysema. Asthmatic attack	2200	200	+2	-8					10	12
6 Emphysema. Asthmatic attack	1900	300	+4	-3					14	14
7 Emphysema	3200	225	-1	-6	-3	-9		6.5	7	
8 Emphysema	2700	300	-2	-4	-2	-6		6		
9 Emphysema	2800	270	-1	-4						
10 Emphysema (primary pulmonary arterial sclerosis)	1700	200	+2	-8				14		

\* All intrapleural pressures taken in mid axillary line in 5th interspace.

averaged 5.5 cm of water by the White method. The intrapleural pressure of the same patients when moderate expiratory effort was made against the closed glottis averaged +8 cm of water. Venous pressure in the normal individuals increased to 18 cm of water. Emphysematous patients in whom there was no evidence of respiratory distress usually were found to have, during expiration, intrapleural pressures which were nearer to atmospheric pressure than the normal. In these patients the venous pressures averaged 7 cm of water, which is at the upper limit of normal range. One patient who suffered respiratory distress from an asthmatic attack and whose vital capacity was 50 per cent of his estimated normal had an intrapleural pressure of +4 to -3 cm of water with tidal air of 300 cc. His venous pressure was 14 cm. Two other patients during mild asthmatic attacks had intrapleural pressures of +2 to -8 and -1 to -6. The venous pressure in these patients was 10 and 7 respectively. After subsidence of the attack the venous pressure decreased somewhat but did not become less than 6 cm of water.

The intrapleural pressure in chronic emphysema was found to vary over different areas of the lung. Thus, in two cases over the upper lobe it was less negative than over the base as noted from the following figures



TABLE II  
*Variation of intrapleural pressure over different areas of the chest in emphysema*

Patient number	Tidal air	Intrapleural pressure lower border of scapula		Upper border of scapula	
		Inspiration	Expiration	Inspiration	Expiration
	cc	cm water	cm water	cm water	cm water
10	300	-6	-2	-3	+1
11	350	-5	-3	-3	+2

The venous pressures of a group of asthmatics entering the hospital were taken at least twice every day and occasionally, when the attacks were frequent, more observations were made. It was found that the venous pressures rose during the attacks and fell afterwards. In those asthmatic patients whose chest presented very little evidence of emphysema the venous pressure fell immediately, but in cases where a marked emphysema was present the venous pressure dropped more slowly. Sometimes several days were required for a return to a normal range.

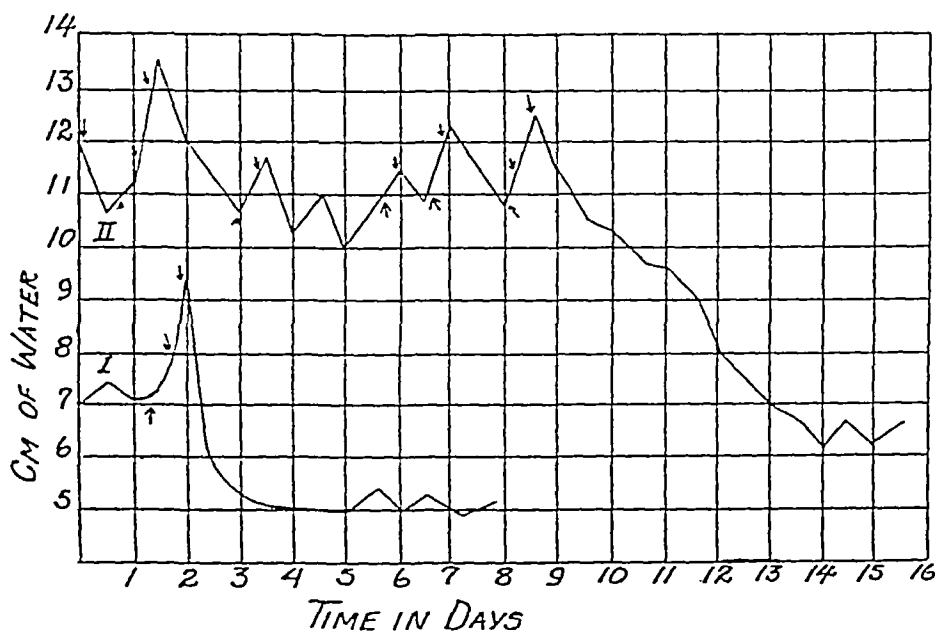


CHART I

I RECORD OF THE VENOUS PRESSURE OF AN ASTHMATIC INDIVIDUAL WHO HAD ONLY A MILD DEGREE OF EMPHYSEMA

The venous pressure immediately dropped to normal after an asthmatic attack. Arrow up indicates onset of attack. Arrow down indicates an injection of adrenalin.

II RECORD OF THE VENOUS PRESSURE IN AN ASTHMATIC INDIVIDUAL WITH EMPHYSEMA

The venous pressure returned to normal much more slowly after the attacks ceased than in number I. Arrows up indicate attacks. Arrows down indicate adrenalin.

The relative circulation rate was determined in normal subjects and in emphysematous patients by the method devised by Hamilton, Moore, Kinsman, and Spurling (7). A dye, phenoltetraiodophthalein sodium (6 milligrams per kilogram of body weight), was injected into a cubital vein, and blood was simultaneously collected from the brachial artery of the other arm into small glass tubes containing heparin, which were mounted about the periphery of a moving drum. A number 20 short beveled Luer needle was used for the arterial puncture and the blood was run directly into the tubes. The time of the injection of the dye was

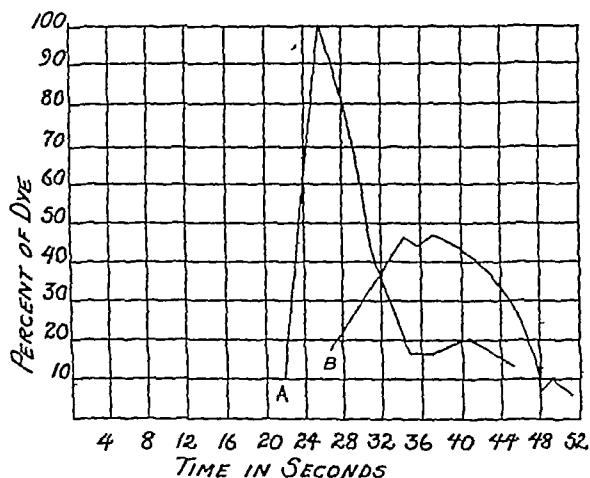


CHART II

CONCENTRATION WAVE OF DYE INJECTED INTO THE MEDIAN CUBITAL VEIN OF ONE ARM AND REMOVED FROM THE BRACHIAL ARTERY OF THE OTHER

(A) Normal man Pulse rate 67 (B) Asthmatic with moderate obstruction to breathing Pulse rate 70 The standard for the curve was the maximum concentration of dye in the serum of the normal

electrically recorded on the kymograph by a break switch attached to the injecting syringe. Time was recorded by a chronograph. The tubes containing the blood were centrifuged and a drop of 10 per cent sodium hydroxide was added to the serum from each tube to bring out the purple color of the dye. In this way the time required for the dye to reach the first tube was determined. By comparison of the dye in the succeeding tubes with a known standard, a "concentration wave" of the dye as it passed the collecting point in its first circuit could be plotted as described by the originators of the method.

They found the normal circulation time to be approximately 23 seconds with a pulse rate of 72. In two normals we found the circulation time to be 23 and 22 seconds with a pulse rate of 74 and 76. In one patient with extreme emphysema and a pulse rate of 72 the circulation time was 28 seconds or 5 seconds above the average normal. In three patients with moderate emphysema the circulation time was 24, 23, and 25 seconds respectively, the pulse rates, however, were more rapid. In two patients who had emphysema without demonstrable functional changes the circulation time was well within the normal range.

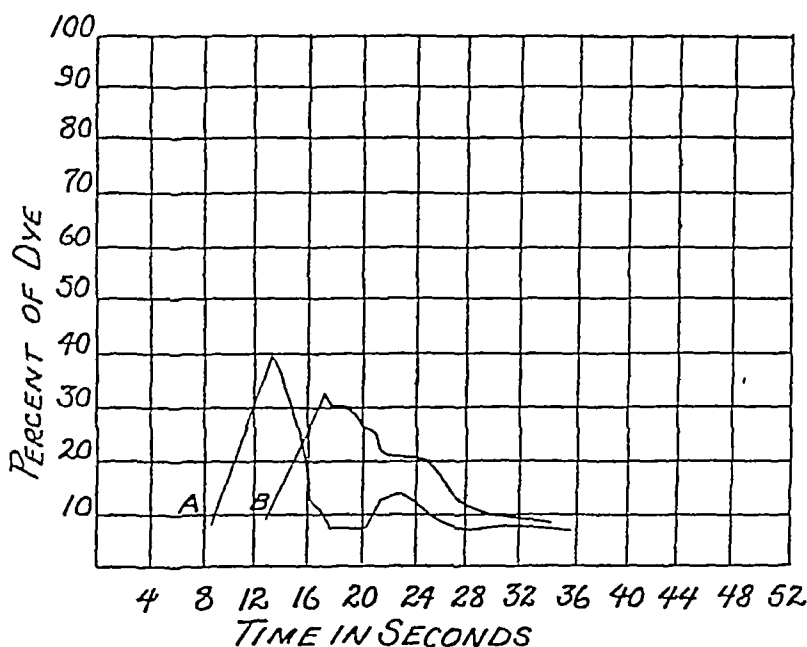


CHART III

CONCENTRATION WAVE OF DYE INJECTED INTO THE FEMORAL VEIN OF A DOG AND REMOVED FROM THE RIGHT HEART

(A) Dog in normal state under amytal anesthesia (B) Same dog made emphysematous by a ball valve in trachea. A prolongation of 4 seconds in the appearance of the dye in the emphysematous animal.

The circulation time studies were carried out in dogs and an attempt was made to determine the point of the relative obstruction to the circulation that occurs in high grade emphysema. The method of injecting the dye (500 mgm. in all dogs) and collecting the blood was similar to that employed with patients. In one group of dogs the dye was injected into the femoral vein of one leg and the blood collected from the femoral artery of the other leg. In the next group the dye was injected into the femoral vein and collected from the right heart. In the third group the dye was injected into the right heart and collected from a femoral artery. Amytal anesthesia was used. Glass arterial cannulae were used when

blood was collected from the femoral arteries and the blood was directed into the collecting tubes by a glass tube 10 cm in length. A number 16 Luer needle was used both for injection of the dye into and for collection of blood from the right heart. The cardiac taps were made in the 7th interspace on the right side of the sternum with the needle directed upwards in order to strike the right ventricle. When blood was collected from the right heart it was necessary to have the dog swinging above the collecting tubes with the chest downward.

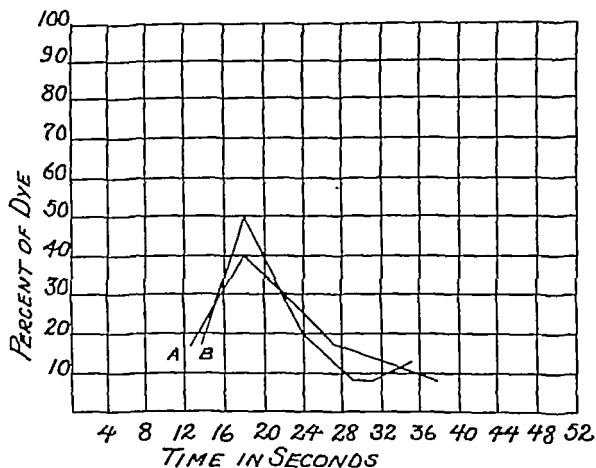


CHART IV

CONCENTRATION WAVE OF DYE INJECTED INTO THE RIGHT HEART AND REMOVED FROM THE FEMORAL ARTERY

(A) Emphysematous animal (B) Normal animal. There was no delay in appearance of first tube of dye and the waves were similar in character. Pulse rates were the same.

The dogs were allowed to recuperate and after a period of three to four weeks their lungs were distended by inserting an adjustable valve in the trachea which obstructed expiration (4). An emphysematous condition was thus produced in the animals and the circulation rate was again determined using the same point of injection and collection. A difficulty in the use of the method in some animals arose from the increase in heart rate from the asphyxia which occurred during the production of emphysema. Slowing of the heart rate under amytal anesthesia, however, was the usual result obtained. The dye was injected when the heart rate was normal. The effect of asphyxia on the heart muscle and consequently on the cardiac output could not be determined. Kountz

and Hammuda (8) have shown that slowing of the heart in asphyxia precedes by some time a diminution in cardiac output

There was a prolonged period of circulation time of two seconds or more in the groups in which the dye was injected into the femoral vein and collected from the femoral artery and from the right heart. The concentration curve had a less sharp apex and there was a more prolonged period of high concentration after the production of distention. The group in which the dye was injected into the right heart showed no delay in the time appearance of the dye in the first tube when the animals were made emphysematous.

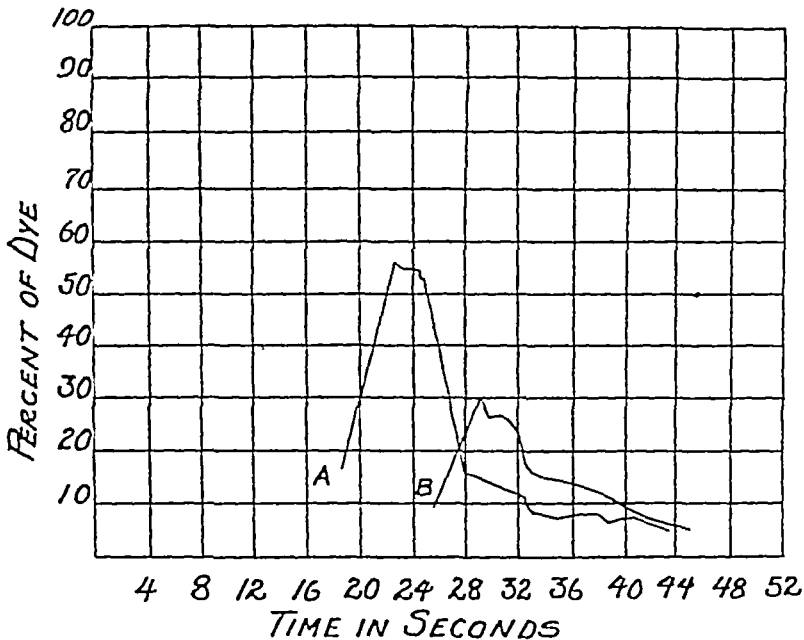


CHART V

CONCENTRATION WAVE OF DYE INJECTED INTO THE LEFT FEMORAL VEIN AND REMOVED FROM THE RIGHT FEMORAL ARTERY

(A) Animal in normal state under amytal anesthesia (B) Animal made emphysematous—under amytal anesthesia. Note prolongation of circulation of 6½ seconds and a change in the concentration curve. Heart rate 90 to 94 respectively.

#### DISCUSSION

It is extremely difficult to separate the various factors operative in the functional pathology of emphysema. Hoover (9) and others have elaborated on the important effects of decreased vital capacity and increased residual air. Friedmann and Jackson (1) were first to question the authority of those who believed that the respiratory difficulties provided the entire explanation for the hemorespiratory incompetence. These authors believed that there is impairment in the circulation in emphysema but they, like subsequent investigators, mentioned only the possibility of blockage of the circulation in the lungs.

We found by clinical and experimental observations on the venous and intrapleural pressures in obstructive emphysematous conditions and by determination of the circulation rate that there is a relative obstruction to the flow of blood into the thorax when the vital capacity is low. Many other observations support this view.

It is known that the heart decreases in size during the Valsalva experiment. If the obstruction were in the lungs, this experiment should cause an increase in the size of the heart. The inference must be that the circulation is obstructed before it reaches the right heart. We have found an increase in venous pressure and intrapleural pressure during this experiment. Furthermore, Dolley and Weise (10) have shown by increasing the intrapleural pressure with a closed pneumothorax that the flow of lymph into the chest may be diminished 50 per cent.

The same phenomena as observed during the Valsalva experiment have been shown to exist in emphysematous patients but to a less striking degree. In general there is a parallelism between venous and intrapleural pressures in emphysematous patients with exacerbations and remissions of respiratory distress and both pressures remain above normal in extreme cases. Furthermore, Alexander, Luten, and Kountz (11) have shown that there is no more tendency to right sided cardiac hypertrophy in long standing emphysematous patients than in other people of the same age group. The above facts indicate that the load on the right heart is probably not increased in emphysema and therefore the theoretical resistance in the lesser circulation probably does not exist.

The experiments which have been performed by others on excised lungs and which show an increase in perfusion pressure when intrabronchial pressure is increased are not of value in interpreting the actual conditions in the intact thorax. Any type of pressure exerted on the excised lungs would not be compensated by other pressure relations. There is no intrapleural pressure effect and the circulation is not intact.

By direct study of the circulation in dogs the physiological principles involved in emphysema became more apparent. It was found that as distention of the lungs in the experimental animal was produced there was first a drop and then a rise of the intrapleural pressure. This has recently been confirmed by Brill and associates (12). A rise of the venous pressure accompanied a rise of the intrapleural pressure. An increase in the time of the circulation rate was constantly found. In the experiments the circulation rates seem to offer evidence concerning the site of obstruction but must be interpreted with caution. A prolonged period of 2 or more seconds occurred between the points of injection and recovery when the point of entrance to the thorax intervened and there was an increase in dilution of the dye before it was collected. This dilution is explained by greater diffusion of the dye due to a condition of relative venous stasis. On the other hand, when the dye was injected

into the right heart and carried only through the pulmonary circulation before it was recovered at the periphery no variations in the time and only a slight variation in the concentration wave of the dye occurred after the dogs were made emphysematous

It seems impossible to explain the results on the basis of obstruction in the pulmonary circuit. If this were the site of block, the dilution should have been equally as great when the dye was injected into the right ventricle. Dilution under these circumstances, however, may have been influenced by factors other than circulation time, and the relative volume and rigidity of the venous bed on the one hand and of the right ventricle on the other introduce influences which cannot be accurately evaluated.

It has been shown that in the absence of heart failure or direct obstruction to venous return the venous pressure is a rough index to the intrapleural pressure. The peripheral venous pressure has been shown to rise appreciably during an asthmatic paroxysm. The rate of return to normal varies. In patients who have elastic lungs the venous pressure returns to the normal range quickly after the cessation of the asthmatic attack the same as it does after the Valsalva experiment. In patients who have marked emphysema with asthma the fall is slow, and the original level may never be reached. We believe that this is due to the relative inelasticity of the lungs when emphysema is present, a feature which is perhaps fundamental to the development of permanent increased intrapleural pressure. The relative high intrapleural pressure over the apex of the lungs in chronic emphysema perhaps accounts for the prominence of veins of the upper portion of the body so characteristic in this disease.

#### SUMMARY

1 The intrapleural and peripheral venous pressures are found to be increased in functional obstructive emphysema. In the experimental animal with obstruction to expiration in the trachea there is an upward trend of both pressures after the lungs are distended. Either clinically or experimentally a rise in the intrapleural pressure is found to reflect directly upon the venous pressure.

2 When dye is injected into a peripheral vein and removed from an artery in obstructive emphysema or in an experimental animal whose lungs were distended to a point of full inspiration an increase in the circulation time was noted. Dye injected into the right heart in the experimental animal and removed from an artery showed no delay in the circulation time although some evidence existed to indicate that the right heart did not empty itself as quickly as it did under normal circumstances.

3 It is suggested that although there may have been some obstruction to a normal amount of blood passing through the lungs, the chief obstruction was at or peripheral to the entrance of the blood into the thorax. Certainly the delay occurred before the blood reached the right heart.

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# QUANTITATIVE ASPECTS OF IRON DEFICIENCY IN HYPOCHROMIC ANEMIA

(THE PARENTERAL ADMINISTRATION OF IRON)<sup>1</sup>

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Evidence has been accumulating in recent years which suggests that certain forms of hypochromic anemia are primarily due to a deficiency of iron (1), (2), (3) In deficient conditions, generally, the ultimate rôle played by the deficient substance is unknown This is especially true in the vitamin deficiencies In the study of the quantitative aspects of iron deficiency the information yielded by the oral administration of iron is of only limited value, because the greater part of the iron so administered leaves the body in the feces When iron is administered parenterally it is reasonable to believe that only an excess will be excreted Since much the greater part of the iron in the body is contained in the hemoglobin, and the hemoglobin is the substance in the body apparently most affected in hypochromic anemia, and since the hemoglobin is easily available for accurate determination, it appears practical to study quantitatively this type of deficiency by means of the parenteral administration of iron

The purpose of the present study is two fold first, to describe the dosage of iron which may be given parenterally in hypochromic anemia, and to compare it with the dosage by mouth, secondly, to determine the fate of iron administered parenterally, and establish a quantitative basis for the better knowledge of this type of deficiency This will lead, it is hoped, to a better understanding of the quantitative aspects of other deficiency disorders

The study presented here has been made upon seventeen consecutive cases of hypochromic anemia treated with iron administered parenterally A summary of the clinical data is given in Table 1 The cases were of types known to respond well to iron therapy by mouth The anemia was either of the idiopathic hypochromic type or was due to chronic blood loss, inadequate diet, previous pregnancies, or combinations of

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<sup>1</sup> The expenses of the present investigation were borne in part by grants from the J K Lilly gift to the Medical School of Harvard University

TABLE 1  
Summary of clinical data

Case number	Age	Sex	Diagnosis	Factors etiologic in the anemia
1	38	F	Idiopathic hypochromic anemia with hypochlorhydria	Hypochlorhydria, chronic blood loss, inadequate diet, multiple pregnancies
2	27	F	Hypochromic anemia, miscarriage	Chronic and subacute blood loss, inadequate diet
3	32	F	Hypochromic anemia, fibroma uteri	Chronic blood loss, inadequate diet
4	25	F	Idiopathic hypochromic anemia with hypochlorhydria, chronic menorrhagia	Hypochlorhydria, chronic blood loss, inadequate diet
5	35	F	Idiopathic hypochromic anemia with achlorhydria (Plummer-Vinson syndrome)	Achlorhydria, inadequate diet
6	52	F	Idiopathic hypochromic anemia with achlorhydria, bleeding hemorrhoids	Achlorhydria, chronic blood loss, inadequate diet, multiple pregnancies
7	32	F	Hypochromic anemia, bleeding hemorrhoids	Subacute blood loss, inadequate diet, recent pregnancy
8	39	F	Idiopathic hypochromic anemia with achlorhydria, chronic menorrhagia	Achlorhydria, chronic blood loss, inadequate diet
9	41	F	Chronic hypochromic anemia with achlorhydria, pregnancy	Achlorhydria, pregnancy, chronic blood loss
10	17	F	Hypochromic anemia, abortion	Subacute blood loss, inadequate diet
11	61	F	Idiopathic hypochromic anemia with achlorhydria (Plummer-Vinson syndrome), rheumatic heart disease	Achlorhydria, inadequate diet
12	22	F	Chronic pyelitis, ischio rectal abscess, chronic mastoiditis	Inadequate diet (chronic sepsis)
13	31	F	Idiopathic hypochromic anemia with achlorhydria	Achlorhydria, inadequate diet, recent pregnancy
14	28	F	Hypochromic anemia	Inadequate diet, recent pregnancy
15	25	M	Hypochromic anemia, Banti's disease, splenectomy	Subacute blood loss (Banti's disease)
16	56	M	Hypochromic anemia, bleeding peptic ulcer	Chronic blood loss, inadequate diet
17	67	F	Idiopathic hypochromic anemia with hypochlorhydria, bronchiectasis, tertiary lues	Hypochlorhydria, inadequate diet (sepsis)

these factors. Complications which significantly inhibit blood formation were absent, such as severe sepsis, severe damage to organs, and carcinoma. Adequate control periods before therapy was instituted were regarded as essential in such a study, and were made in each case.

#### METHODS

Blood was taken daily from the ear for reticulocyte counts during the period of close observation. As a rule, every second or third day venous blood was taken for complete blood studies. The blood counts were made with pipettes and counting chambers certified by the U S Bureau of Standards. The hemoglobin was determined with the Sahli Haemometer, which had been standardized by determinations of the oxygen capacity of the blood using the Van Slyke apparatus. One hundred per cent hemoglobin was taken as the equivalent of 15.6 grams per 100 cc. of blood, or 21 volumes per cent oxygen capacity. Blood volumes were determined upon five cases by the vital red dye method of Rowntree, Brown and Roth (4).

Except in two instances which will be indicated, iron citrate green (N N R) in various dilutions, usually in 10 per cent solution, was used for daily intramuscular or subcutaneous injection. This is a neutralized solution of iron and ammonium citrate with 0.5 per cent quinine and urea hydrochloride as a local anesthetic. The iron and ammonium citrate in this preparation contains approximately 16 per cent of metallic iron. Solutions containing 10 per cent iron and ammonium citrate were used as a rule, but sometimes more dilute solutions were administered. It was given usually in a dose corresponding to from 8 to 32 mgm metallic iron at each injection. Iron and ammonium citrate (brown scales<sup>1</sup>) was used orally, usually in a dose of 6 grams daily, corresponding to approximately 1 gram of metallic iron.

Iron in doses of 16 to 32 mgm a day, given parenterally, is very close to the maximum amount of iron tolerated by man. It is attended by disagreeable symptoms, sometimes severe and possibly dangerous. Severe local pain usually follows the intramuscular injection, lasting about twenty-four hours, and much longer if the solution is not neutralized. Immediately following the injection, and for about thirty minutes afterwards, the patient experiences a disagreeable feeling of general warmth, palpitation and sometimes a pressure sensation in the region of the precordium, nausea and frequently vomiting. After the administration of 48 mgm and 80 mgm in two patients, respectively, there were much more severe reactions. Five minutes after the injection there was flushing of the face, engorgement of the neck veins and an appearance of intense anxiety. Vomiting occurred much as it does after the administration of apomorphine. The pulse was rapid and full, the heart sounds loud, and there was moderate hyperpnea. Precautions were especially taken that the solution should not be injected directly into a vein, and yet the rapidity of onset of symptoms suggested this possibility. After one hour the disagreeable symptoms had nearly disappeared. It was thought that such large doses of iron were distinctly dangerous. The injection of 8 mgm of iron a day was attended by more moderate symptoms of the above nature.

<sup>1</sup> No significant difference has been found in the effectiveness of brown scales and green scales given orally to patients with hypochromic anemia.

*Comparison of the parenteral dosage with the oral dosage of iron*

The first ten cases, after the necessary control period, were given iron parenterally each day for about ten days. On the day following the last injection of iron, the daily oral dose of iron and ammonium citrate was commenced. This method of investigation has been employed successfully (5), (6) to compare, by means of reticulocyte responses, the potency of substances affecting the blood. Following the subsidence of a rise of reticulocytes due to the uniform daily administration of the first substance, a rise of reticulocytes due to the uniform daily administration of the second substance is proof that this second substance, in the quantity given, is more potent than the first in the quantity given, provided that the two are similar in their quality. A summary of the hemoglobin and reticulocyte responses to both parenteral and oral iron therapy is given in Table 2.

Case 1 received a commercial preparation entitled "endocolloidal iron" said to contain 8 mgm of metallic iron in the daily dose. No hemoglobin or reticulocyte response resulted from these injections in this patient. Case 2 was given a commercial preparation of a "colloidal" solution of ferric hydroxide in at least double the maximal dosage recommended by the manufacturers, and attained a moderate reticulocyte rise and a very slow hemoglobin rise. It is possible that the iron given to Case 1 was not in an available form for immediate hemoglobin building, but was merely stored.

The ten cases may be divided into three groups: five cases receiving an equivalent of 8 mgm metallic iron daily, two cases receiving 16 mgm daily, and three cases receiving 32 mgm daily. An illustrative case is shown in Figure 1. Table 3 is a summary of the hemoglobin responses in the ten cases and shows the average percentage of hemoglobin rise per day in the three groups. The smallest average daily rise of hemoglobin occurred with the five patients receiving the smallest amount of iron, namely, 8 mgm; the largest average rise of hemoglobin occurred with the group receiving the largest amount of iron, 32 mgm. The average daily rise of hemoglobin of all ten cases during the oral administration of iron was 1.3 per cent, a somewhat smaller rate than for the group receiving 32 mgm of iron parenterally, which was 1.9 per cent. This would seem to indicate that 32 mgm of iron given parenterally was a somewhat more effective dosage than 1,000 mgm of iron given orally.

On the other hand, when the reticulocyte responses are considered, a different state of affairs exists. It will be seen from Table 2 that in all of the cases, except Case 5 and Case 10, a definite reticulocyte response was observed during the period in which iron was administered orally. In Case 5 no reticulocyte counts were made in the second period. In Case 10 the oral administration of iron was commenced before there was a complete subsidence of the reticulocytes, which may have masked a small

## Daily administration of iron parenterally

Days of treatment	8 mgm intra-venously		8 mgm intra-venously		8 mgm intra-muscularly		8 mgm intra-muscularly		16 mgm intra-muscularly		16 mgm intra-muscularly		32 mgm intra-muscularly		32 mgm intra-muscularly	
	Case 1		Case 2		Case 3		Case 4		Case 5		Case 6		Case 7		Case 8	
	Hemo-globin	Reticulo-cytes	Hemo-globin	Reticulo-cytes	Hemo-globin	Reticulo-cytes	Hemo-globin	Reticulo-cytes	Hemo-globin	Reticulo-cytes	Hemo-globin	Reticulo-cytes	Hemo-globin	Reticulo-cytes	Hemo-globin	Reticulo-cytes
0	28	0.8	36	4.8	22	0.8	26	1.2	34	2.0	22	2.0	34	2.8	19	2.0
2	29	0.8	36	5.1	22	1.2	26	1.2	36	2.0	22	2.0	37	2.4	20	2.6
4	30	1.0	35	4.4	21	4.2	27	3.0	35	2.4	23	2.6	37	5.2	24	6.2
6	32	0.4	35	7.4	22	2.6	27	1.4	33	3.2	25	3.2	39	5.2	29	6.0
8	26	0.4	37	4.0	24	5.0	28	1.2	30	3.0	25	1.8	41	4.8	29	6.0
10			40	4.4	26	3.0	30	2.4	31	1.4	25	2.0	42	3.6	30	7.2
12					27	3.2			35							
18																

## Daily administration of iron orally

	120 mgm		250 mgm		1000 mgm		1000 mgm		1000 mgm		1000 mgm		1000 mgm		1000 mgm	
	120 mgm		250 mgm		1000 mgm		1000 mgm		1000 mgm		1000 mgm		1000 mgm		1000 mgm	
	Hemo-globin	Reticulo-cytes	Hemo-globin	Reticulo-cytes	Hemo-globin	Reticulo-cytes	Hemo-globin	Reticulo-cytes	Hemo-globin	Reticulo-cytes	Hemo-globin	Reticulo-cytes	Hemo-globin	Reticulo-cytes	Hemo-globin	Reticulo-cytes
2	27	2.6	41	5.0	30	5.4	32	2.8			27	2.4	41	5.6	32	7.0
4	33	8.2	42	7.0	26	4.8	35	3.8			29	5.0	41	6.8	34	10.4
6	41	4.0	45	8.2	30	8.0	37	2.6			30	5.6		10.0	35	6.2
8	45	4.0	50	5.8	39	7.0	39	2.2			34	4.6	46	8.4		
10	38	0.6			52	2.4	41	3.2			37	3.0		2.0	45	5.2
12	50	0.4			58										53	3.0
14	58	0.4					47								53	3.6
16	68	0.2													53	1.4
18	72	0.2	62				52									
20	72	0.2							50		47		54		51	71

Note The oral administration of iron was commenced on the day following the last injection of iron For example, in Case 1, the second day of treatment with the oral administration of iron is the tenth day since treatment first started

second reticulocyte response With these two exceptions, the data indicate that the oral dosage of iron in each case was more effective than the parenteral dosage, and it may be concluded from this reticulocyte data

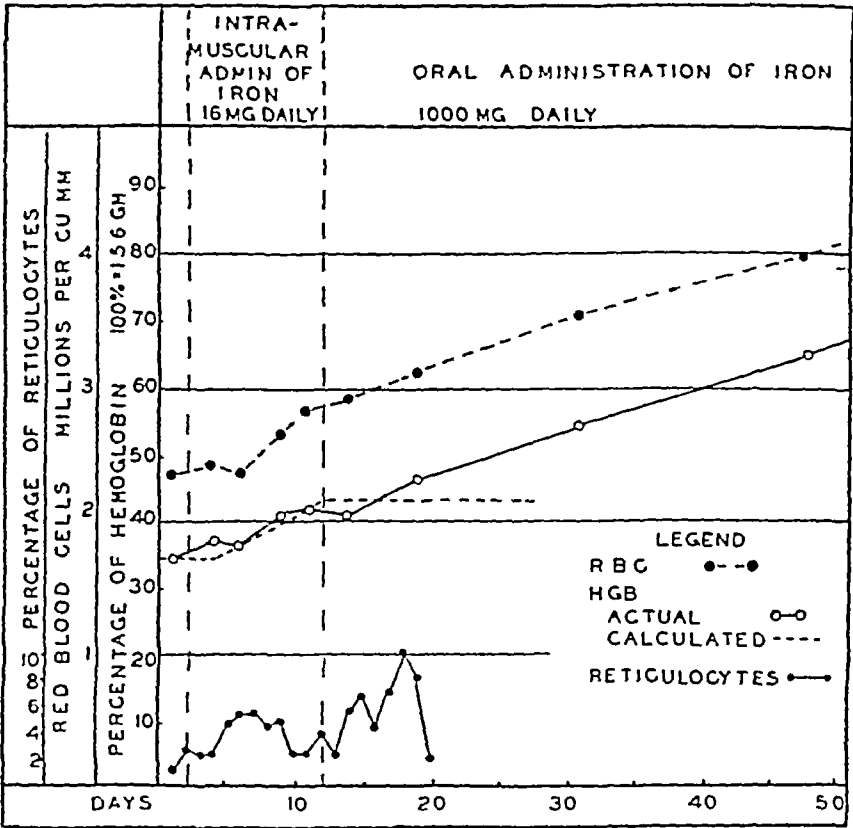


FIG 1 CASE 7, ILLUSTRATING THE RESPONSE OF THE RETICULOCYTES, THE HEMOGLOBIN AND THE RED BLOOD CELLS FIRST TO IRON ADMINISTERED INTRAMUSCULARLY IN SMALL DOSAGE, SECOND TO IRON ADMINISTERED ORALLY IN LARGE DOSAGE

The second reticulocyte "peak" is indicative of a greater effect of the oral dosage of iron Note the close correlation between the actual hemoglobin rise and the rise which is calculated from the total amount of iron given to the patient by injection (see text)

TABLE 3

Comparison of the mean rates of hemoglobin rise following first parenteral and then oral iron administration in 10 cases of hypochromic anemia

	Parenteral iron administration			Oral iron administration
Amount of metallic iron daily, mgm	8	16	32	837
Number of cases	5	2	3	10
Mean hemoglobin per cent rise per day	0.3	0.5	1.9	1.3
Extremes of hemoglobin per cent rise per day	0-0.6	0.5-0.5	0.8-2.0	0.6-2.8

that a dose of 1,000 mgm of metallic iron a day given orally is somewhat more effective than a dose of 32 mgm given parenterally. However, from the information furnished by the rate of the hemoglobin rise the opposite conclusion may be drawn. Further information may be gained by the application of a more refined test for the adequacy of the blood response to iron administration.

From data to be published (7), certain standards for satisfactory hemoglobin and reticulocyte responses following iron in hypochromic anemia have been established. The standard for the hemoglobin response after iron in hypochromic anemia has been determined from the mean of the hemoglobin responses of eighty cases of hypochromic anemia during the oral administration of iron. This standard is about 2 per cent of hemoglobin per day when the initial hemoglobin level is below 50 per cent, and thus in a case of hypochromic anemia a very rapid hemoglobin regeneration and, therefore, an adequate dose of iron is necessary to meet the requirement. The standard for the reticulocyte response has been adopted from data given by Minot and Heath (3) who have shown that the height of the reticulocyte response in hypochromic anemia, after adequate iron therapy, is inversely proportional to the initial level of the red blood cells per cu mm and the hemoglobin percentage. The response of the blood in a case of hypochromic anemia may be considered as a mean percentage of the expected response based upon these standards for hemoglobin and reticulocytes.

Table 4 gives the percentage of the expected response of the hemoglobin and reticulocytes of all seventeen cases based upon these standards. The lowest mean response was obtained with the smallest dose of iron

TABLE 4

*The response of the hemoglobin and the reticulocytes after iron therapy*

(The figures given are a mean per cent of the expected rise of hemoglobin and reticulocytes based upon arbitrary standards)

	Parenteral iron administration			Oral iron administration	
	8	16	32	100-200	1 000
Amount of metallic iron daily, mgm	8	16	32	100-200	1 000
Number of cases	5	2	10	16	38
Mean per cent of expected response	25	40	85	82	89
Extremes of mean per cent of expected response	3-39	26-54	50-130	35-165	40-195
Per cent of cases attaining a mean per cent of expected response of more than 100			40	19	42

given parenterally, namely, 8 mgm daily. The highest mean response, 85 per cent of the expected, standard response, was obtained in the cases receiving the largest dose of iron parenterally. This response compares



favorably with the response of an additional group of cases of hypochromic anemia receiving large amounts (6 grams daily) of iron and ammonium citrate orally. It will be seen in Table 4 that the mean response of the cases receiving 100 to 200 mgm of iron orally was only slightly smaller than that of the cases receiving 1,000 mgm orally, but as has been explained elsewhere (7) the oral dose of 1,000 mgm of iron a day is to be regarded as a much more effective dosage than the smaller amounts, when individual patients are concerned. A dose of iron and ammonium citrate by mouth containing less than 100 mgm of iron daily may produce a satisfactory blood response in an occasional patient, but is much more apt to result in an unsatisfactory response or no response. Among the cases receiving large doses of iron parenterally and 1,000 mgm orally, a similar proportion of each group attained responses of over 100 per cent. 40 per cent of the ten cases receiving 32 mgm or more of iron parenterally attained hemoglobin and reticulocyte responses of over 100 per cent, and 42 per cent of the thirty-eight cases receiving 1,000 mgm of iron by mouth attained responses of over 100 per cent.

*It is therefore considered that a daily dose of 1,000 mgm of metallic iron given orally in the form of iron and ammonium citrate is approximately equivalent in its blood-building power to a daily dose of 32 mgm of metallic iron given parenterally in the form of iron and ammonium citrate to patients with hypochromic anemia.*

The experiments, of course, bring out a tremendous difference in the mass of metallic iron given by the two routes. This is interesting in comparison with pernicious anemia, in which it has been observed that the effective amount of potent material, when injected, is many times smaller than when given by mouth (8), (9). One thousand mgm of iron orally is the customary daily dose when iron and ammonium citrate is employed in this clinic, and represents a dose of 6 grams of the salt which is divided into three portions. As little as 85 mgm of iron orally has occasionally, but not as a rule, been found to be very effective (7). On the other hand, a dose of 2,000 mgm of iron daily has occasionally been found necessary to produce a maximal response, particularly in individuals who have low acidity of the gastric contents. The kind of iron administered orally is also of considerable significance in judging the dosage. The necessary dose of reduced iron is very high (1 to 10 grams) (10), (11), and that of organic iron preparations is probably also high (12). Reimann and Fritsch (13) have demonstrated that ferrous salts are more effective than ferric salts. They have obtained undoubtedly good results with as little as 22 to 100 mgm of iron in the form of ferrous chloride given daily by mouth.

The practical aspects of the oral and the parenteral administration of iron to patients is a problem of an entirely different nature. Iron salts are very cheap, and there is no financial objection to the use of large doses

by mouth, as there is to the use of large doses of liver extract in the treatment of pernicious anemia. Indeed, the actual expense to the patient of intravenous injections of ineffective doses of iron, which are recommended by some physicians, must be absurdly high as compared with the low cost of a simple and very potent iron salt given by mouth, such as iron and ammonium citrate or ferrous carbonate. However, this is not as important as the discomfort to the patient of administering iron parenterally. Thirty-two mgm is at least double the maximum dose of green iron citrate recommended in New and Non-Official Remedies, and the data presented in this paper indicate that even this amount daily is not an optimal quantity for many cases. This dose intramuscularly is attended by severe local symptoms lasting about twenty-four hours, and also by general symptoms of a very disagreeable nature which may be dangerous, as has been described. It is therefore considered that, except in rare instances, the administration of iron by the parenteral route should be avoided.

#### *The utilization of iron administered parenterally*

In studying the data for the first ten cases, it was discovered that the calculated amount of iron in the hemoglobin gained bore a close relationship to the amount of iron injected. In order to determine this relationship more accurately, it was necessary to give parenteral injections for a number of days, followed by a control period in which no iron was given, and in which the complete rise of hemoglobin could be observed. Table 5 is a summary of the results obtained in seven cases. Cases 11, 12 and 13 were given several courses of iron administered parenterally. In all instances there was an appreciable rise of reticulocytes. In all but Case 17 there was a hemoglobin response. In Case 17 there was moderate sepsis present in the form of bronchiectasis, and poor co-operation by the patient necessitated giving only a small amount of iron. In Case 12 pyelitis accompanied by a low grade fever prevented as rapid a hemoglobin response as is ordinarily observed, but a satisfactory result was at length attained.

In order to determine the total amount of hemoglobin formed as a result of iron treatment it was necessary to know the patient's blood volume. This was determined in Cases 11, 12, 13, 14 and 15. The average blood volume per square meter of body surface of these five cases was used in estimating the blood volume of the other cases. This was 2,423 cc. per square meter of body surface, a figure somewhat lower than that reported by Rowntree, Brown, and Roth (4) for secondary anemia, but probably more desirable for this series, not only because the patients had a greater degree of anemia than those of Rowntree, Brown and Roth, but also for the sake of uniformity of methods.

The patients were all maintained upon diets that did not contain

TABLE 5

The effect of administering to 7 cases of hypochromic anemia iron parenterally Summary of hemoglobin and reticulocyte responses

Days of treatment	Case 11			Case 11 (Continued)			Case 12			Case 13			Case 14			Case 15			Case 16			Case 17		
	Main daily dose of iron	Hemo-globin	Reticulo cytes	Main daily dose of iron	Hemo-globin	Reticulo cytes	Main daily dose of iron	Hemo-globin	Reticulo cytes	Main daily dose of iron	Hemo-globin	Reticulo cytes	Main daily dose of iron	Hemo-globin	Reticulo cytes	Main daily dose of iron	Hemo-globin	Reticulo cytes	Main daily dose of iron	Hemo-globin	Reticulo cytes			
0	48	16	08	32	42	20	27	42	11	30	40	26	33	51	53	1000	30	41	50	31	850	31		
2	mgm	10	22	mgm.	45	26	mgm.	50	22	mgm	41	40	mgm.	51	03	mgm.		57	40	mgm.	31			
4	1m	16	54	1m	47	29	1m.	50	42	1m	44	40	1m	55	00	p.o		23	40	p.o	33			
6	(for 3 days)	10	7.5	(for 13 days)	40	3.3	(for 5 days)	40	14	(for 4 days)	44	34	(for 7 days)	61	00	(for 3 days)	41	92	40	(for 2 days)	55			
8	21	21	34	40	40	4.3	40	61	28	47	22	22	40	60	77	48	47	113	48	53	38			
10	22	22	22	47	47	23	55	55	20	40	11	11	01	01	47	48	48	188	50	53	40			
12	24	24	12	47	47	10	50	50	10	47	08	00	01	12	48	48	48	153	53	53	41			
14	61	23	04	10	20	2.5	10	48	19	32	47	00	01	12	48	48	48	153	53	53	41			
16	mgm.	23	14	mgm.	53	11	mgm.	53	41	mgm.	47	10	1,000	63	17	30	40	38	50	55	42			
18	1m	20	50	1m.*	53	12	(for 20 days)	53	28	1m.	47	88	mgm.	61		mgm	50	34	51	60	11			
20	(for 4 days)	29	58	50	50	09	50	52	30	(for 9 days)	40	87	p.o	70		mgm	50	84	57	60	42			
22	31	31	18	58	58	10	54	52	24	55	85	85	55	55	85	55	55	111	57	58	44			
24	33	33	17	60	60	08	54	50	10	57	80	28	57	57	74	57	57	74	58	44	41			
26	31	31	14	60	60	08	54	50	10	57	80	28	57	57	74	57	57	74	58	44	41			
28	31	31	14	60	60	08	54	50	10	57	80	28	57	57	74	57	57	74	58	44	41			
30	31	31	14	60	60	08	54	50	10	57	80	28	57	57	74	57	57	74	58	44	41			
32	35	35	00	170	60	10	170	60	10	1,000	63	12	70	70		mgm. p.o	57	32	58	44	43			
34	80	35	14	mgm	60	02		60	03		65													
36	mgm	30	10	p.o	60	08		60	05		65													
38	1m	38	10		62	1.8		62	02		65													
40	(for 3 days)	38	17		65	1.2		65	01		65													
42	38	38	17		58	04		60	00		63													
44	38	38	00		59	05		60	07		63													
46	Liver	39	10		62	05		60	07		65													
48	extract	37	11		61	10		60	08		65													
50	(No 313	38	15	1,000	65	08		65	08		65													
52	NN R)	40	20	mgm.	65	08		67	07		65													
54	derived	43	23	p.o	64	05		67	07		65													
56	from 000	44	22		63			70	70		67													
58	grams of liver for 16 days	44	16		60			70	70		67													
		43	24		67																			

\* Vaccination of chronic pyelitis begun on this day

meat Although it was not known whether or not the patients were in iron balance, this was presumed to be approximately the case, provided that the control periods showed no hemoglobin regeneration The amount of iron in hemoglobin may be considered conveniently as 0.3 per cent (14), a figure which checks closely with that obtained from the oxygen capacity of the blood if hemoglobin combines with one molecule of oxygen per atom of iron (15) From these data the total amount of circulating hemoglobin, and also of iron in the hemoglobin, may be easily calculated The total amount of iron gained by the circulating hemoglobin after iron therapy is the difference between the final total amount of iron in the circulating hemoglobin and the total amount before iron therapy If the total amount of iron given to the patient is known, the percentage of utilization of this iron for the building of new hemoglobin is easily ascertained by dividing the grams of iron gained ( $\times 100$ ) by the grams of iron injected

Table 6 gives the results of these determinations upon all the cases excepting Cases 1, 5, and 17 Cases 1 and 17 had no demonstrable rise

TABLE 6  
*The utilization of iron administered parenterally*

Case number	Period of iron injection	Iron in circulating hemoglobin	Iron injected	Iron gained in circulating hemoglobin	Per cent of utilization of injected iron
		grams	grams	grams	per cent
2	1	514	080	084	105
3	1	429	096	108	112
4	1	486	080	123	154
6	1	432	144	146	101
7	1	626	160	128	80
8	1	341	320	225	70
9	1	663	304	211	69
	2	994	256	161	66
10	1	636	224	281	125
11	1	332	144	118	82
	2	450	256	248	97
	3	698	160	137	86
	4	835	416	276	66
12	1	609	136	131	96
	2	740	320	259	81
13	1	582	144	126	87
	2	708	288	388	135
14	1	883	224	231	103
15	1	1 074	209	165	79
16	1	1 137	112	085	76

of hemoglobin and therefore did not utilize the injected iron for reasons which have been explained In Case 5 the number of hemoglobin determinations were insufficient On Figures 1 and 2 the broken lines represent the calculated response of the hemoglobin to the amount of iron

injected, assuming that the hemoglobin began to rise after the second day at a rate of 1 per cent per day. The close relationship of the actual to the calculated gain of hemoglobin is striking, and is especially well illustrated in Figure 2. It is evident that the error involved, however, in determining the percentage of utilization when only small amounts of iron are given is so great that it detracts considerably from the significance of the results.

Case 11, a female with idiopathic hypochromic anemia with achlorhydria and with an associated dysphagia (Plummer-Vinson syndrome), deserves particular attention. The course of the recovery from anemia in this case is shown in Figure 2. Following the third course of iron injections she was given liver extract number 343 (N N R) in a daily amount which was derived from 600 grams of liver. This was given in the belief that such cases may be anemias due not exclusively to iron deficiency, but forms that respond not only to iron but also, to a less extent, to some of the substances present in liver extracts. The patient did have a slight reticulocyte response and a moderate hemoglobin rise during this period of liver therapy which, however, was not sustained. It was thought that since liver extract number 343 (N N R) contains very slight amounts of iron this did not interfere with the observations on the effectiveness of the iron therapy. Indeed, the giving of liver extract to this patient may have removed at least some increments of the anemia which were not directly due to iron starvation, and thus have allowed a more complete response to the iron. It is probable that if certain other extracts of liver, effective in hypochromic anemia, had been given, a greater response would have occurred. However, such extracts are known to contain appreciable amounts of iron which would probably account for the greater response in such a patient.

The average utilization of parenteral iron in all cases was 96 per cent, the extremes being 69 and 154 per cent. Unobserved variations in blood volumes, many of which, it must be recalled, were estimated, and inaccuracies of hemoglobin determination are probably the chief factors which account for these wide extremes. Notwithstanding the errors, the figures indicate an extraordinarily close relationship between the amount of iron injected and the amount gained in the circulating hemoglobin. This is shown graphically in Figure 3. Attention is called particularly to the four encircled dots in this figure, corresponding to the four periods of treatment of Case 11, which show a greater gain of hemoglobin with each increase in the amount of iron administered.

The comparison of the percentage of utilization of iron given parenterally with that of iron given by mouth is interesting. When very small amounts of iron are given by mouth it is possible in a few instances to attain over 50 per cent utilization as determined in this manner (7). However, in the treatment of many cases over long periods of time, and

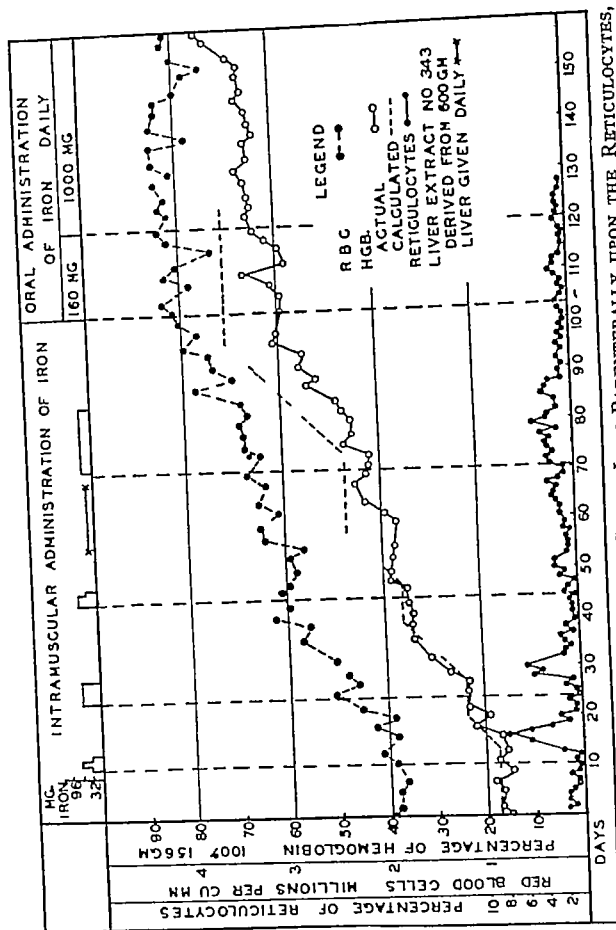


FIG 2 CASE 11 THE EFFECT OF GIVING IRON PARENTERALLY UPON THE RETICULOCYTES, HEMOGLOBIN AND RED BLOOD CELLS

Note the close correlation between the actual hemoglobin rise and the rise which is calculated from the amount of iron given to the patient by injection (see text)

with the usual large doses of iron given by mouth, the utilization is only a little over 3 per cent. No utilization over 100 per cent has yet been observed when iron has been given by mouth. Presumably, a percentage of utilization of "154," which was calculated in Case 4, is the result of unobserved changes in blood volume together with errors in technique. If, however, it were a true value, it would suggest a number of perplexing problems. Whether or not a percentage of utilization of over 100 in certain cases can possibly mean some "stimulating" action of iron, whether or not, in what is believed to be a true iron deficiency, the injected iron is all retained by the body, and whether or not the iron injected merely makes

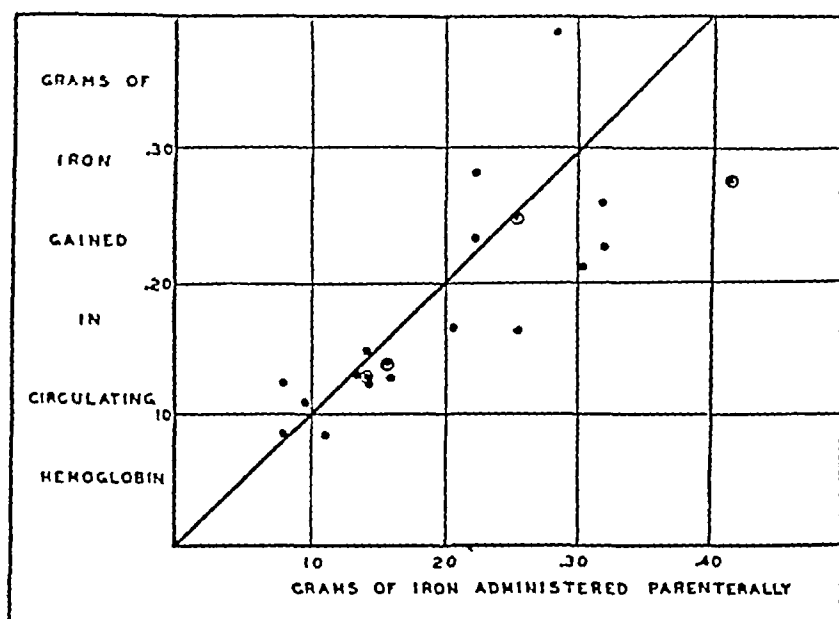


FIG 3 THE RELATIONSHIP BETWEEN THE GRAMS OF IRON ADMINISTERED PARENTERALLY AND THE GRAMS OF IRON GAINED IN THE CIRCULATING HEMOGLOBIN IN 20 OBSERVATIONS UPON 14 PATIENTS

The oblique line represents ideal correlation, or 100 per cent utilization of iron injected. Note the four encircled dots which represent the four periods of iron injection in Case 11, showing a greater amount of iron recovered in the circulating hemoglobin with each greater amount of iron injected.

available other sources of iron in the body for hemoglobin formation are some of the problems awaiting solution. However, it seems justifiable to come to the following conclusion: *the amount of iron (within certain maximum limits) given parenterally in hypochromic anemia corresponds closely to the amount of iron gained in the circulating hemoglobin, and is apparently utilized to a very large extent in the building of new hemoglobin.*

The corollary to this conclusion is that the daily dose of parenteral iron must be of the same magnitude as the amount of iron in the desired daily rise of hemoglobin. A conservative estimation of a satisfactory rise

of hemoglobin, based upon the experience gained from treating many cases of hypochromic anemia, is about 1 per cent of hemoglobin a day. For an individual with a constant blood volume of 5,000 cc, 1 per cent of hemoglobin a day would mean a gain each day of 7.8 grams of hemoglobin containing 23.4 mgm of iron. A daily parenteral dose of this amount of iron is somewhat smaller than the dose of 32 mgm which, as was demonstrated above, is approximately equivalent in effectiveness to a daily oral dose of 1,000 mgm of iron in the form of iron and ammonium citrate.

It is therefore evident that there is no distinct advantage in giving iron parenterally. It is not uncommon to observe a sustained rate of hemoglobin formation of over 2 per cent a day during the oral administration of large doses of iron. Such a rate would generally require more than 32 mgm of iron given daily by injection, and more than could be considered a safe dose to administer at one time. Weekly or biweekly injections of iron, especially when minute doses are given, are to be deplored as practically useless. Perhaps further work will show that a few cases of hypochromic anemia having complications, or intestinal abnormalities, will be benefited by parenterally administered iron when oral administration fails. Also occasionally some psychic factor may prevent the administration of iron by mouth, and then the cautious use of iron parenterally may be found of distinct value.

#### COMMENT

For many years it has been recognized that iron given parenterally is an effective therapeutic agent in certain forms of anemia (16), (17), (18), (19), (20). It is known that the dose of iron so administered must be small, because of toxic effects (21). The fatal dose of iron injected subcutaneously into animals is quite small, and has been estimated to be about 30 to 60 mgm per kilo (22). Starkenstein (23) determined the relation of the toxicity by parenteral administration to that of enteral administration to be, for the ferrous salts, 1/5 to 1/10, and, for the complex iron salts, 1/100. However, the appropriate dose of parenterally administered iron in man has never been clearly defined. Stockman (17) found that a dose of 16 to 32 mgm of iron subcutaneously a day was effective in abolishing anemia in chlorosis. Witts (1) stated that chlorosis could be alleviated by the injection of 1 to 2 grains of iron and ammonium citrate in 5 to 10 per cent solution daily, that is, 10 to 20 mgm of metallic iron daily. Witts, however, criticized strongly the current tendency to employ for parenteral use unofficial preparations of iron containing infinitesimal amounts of iron.

A great deal of work has been done upon the deposition and excretion of injected iron. It may be stored in animals mostly in the liver and spleen (24), (25), (26). It is excreted by the bowel (27) and by the



kidneys (17), (28) Whether or not, in anemic individuals, injected iron is stored, excreted, or utilized in the newly formed circulating hemoglobin has not been satisfactorily demonstrated. Stockman (17) found that in cases of chlorosis which were given iron subcutaneously, iron in increased amounts was found in the urine invariably, and therefore he believed that all the iron was not utilized in the formation of new hemoglobin.

The study of the present experiments inevitably leads to the belief, without establishing the absolute proof, that iron given therapeutically in hypochromic anemia is supplying a deficiency, and is thus being made available for the normal manufacture of necessary hemoglobin molecules. This point of view gives a logical explanation of the etiology of hypochromic anemia, and provides a simple basis for further experimental work upon this condition. This is a much simpler explanation than the one which assumes that iron, like arsenic or copper, has only a "stimulating," "catalytic" or "tonic" effect upon hemoglobin formation. It is very likely that the reticulocyte response, attributed to the effect of iron, may not be a direct one, but one due to the supply of hemoglobin to the young red blood cells. The delay of about four days usually encountered before the appearance of increased numbers of reticulocytes, following the administration of a potent substance in anemia, can be understood if this time is required for certain new molecules necessary to cell growth to be formed. Iron may "stimulate" in this sense the growth of young red blood cells.

It is not believed that poverty of hemoglobin is the only result of such an iron deficiency, although it is likely that the body economy can best spare iron from the hemoglobin molecule before it gives up iron from the fixed tissues of the body. Whipple (29), for example, has shown that it is difficult to exhaust muscle hemoglobin which is closely related to blood hemoglobin. Iron is known to be present in many, if not in all, cells (30), (31) both plant and animal, but the relatively large amount of iron in the red blood cells, and the ease of observing changes in the corpuscular characteristics of the blood, has focussed our attention upon this part of the body. The dystrophy of the nails commonly observed in idiopathic hypochromic anemia with achlorhydria is very possibly the result of a poor supply of iron to the germinative cells of the nail root. One is tempted also to place under this etiology, certain other changes observed in these patients: atrophy of the skin, atrophic changes of the tongue, fissures about the mouth, and perhaps also certain blood capillary changes which lead to easy bruising and menorrhagia. All of these changes have been observed to disappear as the anemia improves during the administration of iron. Therefore a part of injected or assimilated iron may go to supply deficiencies in the body other than in the blood regenerating apparatus. But it is believed that the amount of iron utilized in this way is extremely small as compared to the amount utilized in the manu-

facture of hemoglobin Some of the iron may also be excreted in the urine or feces

Whipple and Robscheit-Robbins (32) have shown a very complete conservation of hemoglobin injected intravenously into dogs, amounting to 90 per cent or even more Moreover, their work suggests that the body can manufacture certain organic constituents of the hemoglobin molecule (pyrrol substances) (33) The fact that liver and kidney are more effective than iron in regenerating hemoglobin in Whipple's dogs with anemia due to chronic blood loss, whereas these substances are often ineffective in regenerating hemoglobin in idiopathic hypochromic anemia, is explainable from several points of view The standard ration employed by Whipple and Robscheit-Robbins contains 20 mgm of iron daily, which they regard as adequate for maintenance and more than enough to make up for daily wastage and for hemoglobin lost by bleeding (34) The substances lacking in these dogs, therefore, are probably organic rather than inorganic In idiopathic hypochromic anemia a deficiency of iron seems to be the chief factor in preventing adequate regeneration of hemoglobin In other forms of hypochromic anemia, especially that following uncomplicated chronic blood loss, the administration of liver may be followed by as satisfactory blood regeneration as when only iron is employed, although the administration of both liver and iron in certain cases is sometimes more satisfactory than either alone (35) In such conditions, as in Whipple's dogs, the substances preventing adequate hemoglobin regeneration may be principally organic Because a utilization of more than 100 per cent of the administered iron has not been demonstrated satisfactorily there seems to be no definite evidence of a "catalytic" influence or "salt effect" of iron in the production of hemoglobin in human anemia

As Whipple has aptly expressed it "iron is an elusive sprite," and in spite of a voluminous literature representing countless experiments, we are far from reaching a final conclusion in problems concerning iron metabolism However, as observations upon patients and upon experimentally produced anemia accumulate, it becomes possible to formulate certain basic principles which aid in the classification and alleviation of anemia The present experiments give evidence to prove that, in what has been considered iron deficiency, the deficient substance, when given by the parenteral route, may be quantitatively recovered in an organized form in the blood stream As our knowledge of chemical processes progresses, the quantitative relationships of vitamins and other necessary food substances to deficiencies will become more accurately known

## SUMMARY AND CONCLUSIONS

1 Seventeen consecutive cases of hypochromic anemia have been studied in reference to hemoglobin regeneration and reticulocyte response following the administration of iron parenterally and orally

2 A daily dose of 1,000 mgm of metallic iron given orally in the form of iron and ammonium citrate is approximately equivalent in its blood-building power to a daily dose of 32 mgm of metallic iron given parenterally in the form of iron and ammonium citrate to patients with hypochromic anemia

3 The amount of iron given parenterally (within certain maximum limits) corresponds closely to the amount of iron gained in the circulating hemoglobin, and is apparently utilized to a very large extent in the building of new hemoglobin

4 Because of the toxicity of iron administered parenterally in adequate dosage, and for practical and economic reasons, it is believed that it is undesirable to give iron parenterally rather than orally in most cases

5 A simple explanation is offered for the etiology and the effectiveness of iron therapy in certain types of hypochromic anemia, namely, that these types of anemia are due to a deficiency, chiefly of iron, preventing adequate hemoglobin formation. The relationship of the deficient substance, iron, to the deficiency itself, which is mainly in the circulating hemoglobin, can be expressed in a quantitative fashion

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# THE ROLE OF CALCIUM, PHOSPHORUS AND VITAMIN D IN PREGNANCY<sup>1</sup>

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Within recent years the importance of calcium and phosphorus in the diet in pregnancy has received considerable attention. In considering the nutritional value of these elements, it was also quite natural that the rôle played by vitamin D should be investigated. The value of these substances has been studied particularly from the standpoint of pathological conditions of pregnancy, viz, osteomalacia and eclampsia. Maxwell, Miles and Feng (1, 2) have made a rather complete study of osteomalacia in China and found that this condition could be relieved by placing the mothers on a high calcium and phosphorus diet supplemented by vitamin D. Hughes et al (3) in India, likewise had excellent results with this same treatment. Also, in the toxemias of pregnancy some interesting observations have been reported from the use of vitamin D and calcium. Richardson (4) reports favorable results in the treatment of eclampsia with large doses of vitamin D, and Minot and Cutler (5) give good evidence that a high calcium diet or calcium salts intravenously will relieve the symptoms of eclampsia.

It seemed advisable, therefore, to make a study of the effect of these three agents in pregnancy, primarily from the standpoint of the transmission of calcium and phosphorus from the mother to the fetus. The primary object of the investigation was to see if the ash content of the fetus could be controlled by the character of the maternal diet, and secondly to determine whether or not there was any perceptible evidence of a drain by the fetus on the calcium and phosphorus content of the maternal bone. There are only two papers bearing on this particular phase of the subject, of which we are aware. Maxwell, Miles and Feng (1, 2) in their work, analysed the fetal bones of stillborns from osteomalacic mothers, and noted some decrease in the normal ash content, and Toverud (6) carried several rats through pregnancy on a low calcium diet and noted a decrease in the ash, calcium and phosphorus content of the maternal femurs.

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<sup>1</sup> Constructed from the thesis presented by Miss Evelyn M Kuhn to the Faculty of the Rice Institute in partial fulfillment of the requirements for the Degree of Master of Arts, June 1932

## METHODS

All rats used in this investigation were raised from our own stock. They were from four to six months old, and had been fed on the *control* diet (see below) up to the time of mating. Immediately after mating, the females were placed on one of the experimental diets throughout the term of pregnancy. The composition of the diets used was as follows:

1 *Control diet* This consisted of the *basic* diet supplemented by fresh green vegetables and fruits, butter, yeast and cod liver oil.

2 *Basic diet* This had the following composition: linseed oil meal, 15 parts, ground barley, 10 parts, wheat flour, 22 parts, dried buttermilk, 15 parts, rolled oats, 15 parts, yellow corn meal, 20 parts, steamed bone meal, 1 part, ground limestone, 1 part, salt, 1 part. On analysis, this diet was found to contain 97 mgm calcium and 50 mgm phosphorus per 10 gram portion.

3 *Deficient diet* This consisted of wheat flour, 22 parts, yellow corn meal, 77 parts, salt, 1 part. On analysis, this diet contained 13 mgm calcium and 20 mgm phosphorus per 10 gram portion.

Some of the pregnant rats which were being fed either the *basic* or the *deficient* diet were also given 0.5 cc of viosterol 250 D<sup>2</sup> daily. Great care was taken to insure the rats getting this full amount of vitamin D. A small portion of the food was moistened with the oil, and this was eaten by the rats before the remainder of the food was given them.

In connection with this feeding of viosterol and the pregnancy of the rat, a very interesting phenomenon was noticed. The pregnant rats on either the *basic* or *deficient* diets without viosterol, mated and delivered their litters with normal expectancy, i.e. on both diets six out of seven rats which had been mated, delivered their litters at the end of the 22 day gestation period. Also the two rats which were maintained on the *control* diet went through their pregnancies without any trouble. These figures represent an actual diagnosis of pregnancy followed by normal delivery in 14 out of 16 attempts at mating.

On the other hand, those rats who were immediately given the 0.5 cc of viosterol after mating did not come through their pregnancies with such regularity. Of 5 rats who were mated and then immediately placed on the *deficient* diet with viosterol, only one (rat number 15, Table I) came to normal term, while of 7 more rats who were also on the *deficient* diet but who did not receive the viosterol except during the last 10 to 14 days of their pregnancies, 5 came to normal term. That is, only six out of twelve rats went through their pregnancies when given viosterol with a diet poor in calcium and phosphorus. Still lower was the percentage of successful pregnancies in the rats fed the *basic* diet with viosterol added. Of 5 rats who were given viosterol during the 22 day period, *none pro-*

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<sup>2</sup> We are greatly indebted to Mead Johnson and Co., Evansville, Ind., for the generous supply of Viosterol 250 D used in this work.

duced any young, and only two rats, out of 10 mated, delivered their litters when given the viosterol only during the last 14 days of pregnancy. This represents only two successful pregnancies out of fifteen attempts. The reason for this seemingly contraceptive action of viosterol, particularly on the *basic* diet, has not been studied by us, nor has it been previously reported as far as we are aware. That this effect could not be one of environmental or climatic conditions is ruled out, inasmuch as the

TABLE I  
*Calcium and phosphorus content of maternal bone ash and of the offspring*

Mother rat					Number of pups per litter	Averages of pups in litters			
Diet	Number	Weight	Cal cium*	Phos-phorus*		Weight	Ash†	Cal cium†	Phos-phorus†
Control	1	grams	per cent	per cent	10	grams	mgm	mgm	mgm
	2	142	34.2	16.2	7	4.85	91.3	13.8	14.1
Basic									
Basic + viosterol	9	172	40.3	17.8	6	6.03	114.3	18.9	14.9
	10	189	38.2	17.5	6	5.90	113.3	19.0	15.0
Deficient	11	117	31.9	14.4	8	4.63	77.8	10.6	13.4
	12	145	33.0	15.0	6	5.08	87.6	11.9	12.9
	13	133	32.6	14.5	8	4.52	75.6	10.5	11.0
Deficient + viosterol	14	205	37.2	17.8	8	5.95	96.5	14.7	14.4
	15	139	38.0	18.0	7	4.56	80.7	12.5	11.8
	16	142	35.7	16.5	6	4.96	85.2	13.1	13.7
	17	141	35.7	17.3	5	5.01	90.8	11.8	13.6
	18	161	36.3	17.6	10	4.72	75.9	12.9	12.2
	19	155	36.3	17.6	11	4.12	72.9	12.4	11.8

\* Calcium and phosphorus content of the ash of the femurs and tibiae

† Average ash, calcium and phosphorus content in the body of the offspring

rats without the viosterol were mating and delivering at the same time that the viosterol-fed rats were failing. No attempt will be made by us to interpret these findings.

Immediately after birth, and before suckling, the litters were removed. Each rat pup was individually etherized, weighed and ashed to constant weight in an electric muffle furnace. The ash was weighed, dissolved in dilute acid. Aliquots of this solution were used for calcium and phosphorus determinations. In analyzing



were dissected out, and were ashed according to the method of Morgulis (7) by heating the bones to 250° C with a glycerole solution of potassium hydroxide. Upon the resulting bone ash, determinations for calcium and phosphorus were made. All calcium determinations were made according to the method of Roe and Kahn (8), and the phosphorus determinations by the method of Fiske and Subbarow (9).

# RESULTS AND DISCUSSION

It is unnecessary to give the individual weight, ash, calcium and phosphorus data on each of the 140 rat pups which resulted from the nineteen pregnancies. Table I gives the average weight, ash, calcium and phosphorus contents of the pups in the individual litters, as well as the analytical data on the corresponding mothers. Table II condenses

TABLE II

*The influence of diet on the calcium and phosphorus content of maternal bone ash and of the offspring*

Mother rat			Averages of pups						
Diet	Cal- cium*	Phos- phorus*	Weight	Ash†	Cal- cium†	Phos- phorus†	Ash per gram weight	Cal- cium per gram weight	Phos- phorus per gram weight
	per cent	per cent	grams	mgm	mgm	mgm	mgm	mgm	mgm
Basic + viosterol	39.2	17.7	5.96	113.8	19.0	15.0	19.1	3.2	2.5
Basic (and control)	34.8	16.9	4.93	90.5	13.4	13.6	18.3	2.7	2.8
Deficient + viosterol	36.5	17.5	4.89	83.7	12.9	12.9	17.1	2.6	2.6
Deficient	32.5	14.6	4.74	80.3	11.0	12.4	16.9	2.3	2.6

\* Average calcium and phosphorus content of the ash of the femurs and tibias.

† Average ash, calcium and phosphorus content in the body of the offspring.

and summarizes the data of the preceding table, giving by diets, the average values obtained on both mothers and pups. It will be noted that in Table II, the two rats fed on the *control* diet are grouped with the six that were fed the *basic* diet. This is permissible since the data show that there is no difference between the mother rats and pups on these two diets, which fact also indicates that at least for the duration of the experiment the basic diet was entirely adequate for the normal maintenance of the mother rat.

The growth-promoting effect of viosterol on the fetus is quite pronounced, as can be seen from Table II. The mothers on the viosterol diet produced larger pups than those on the corresponding diets without viosterol. Likewise, the absolute ash, calcium and phosphorus contents of the pups were greater under the influence of viosterol, and so were the

relative ash and calcium contents (i.e. ash and calcium per gram pup weight). The variation in the relative phosphorus content was not so great, with a tendency to run inversely to the relative calcium content.

The variation in the calcium and phosphorus content of the maternal bones was also quite noticeable. Both groups of viosterol-fed mothers showed higher contents of these two elements than did the mothers not receiving the viosterol. It has already been pointed out that mothers on the viosterol diet produced young who had a greater content of calcium and phosphorus in them, than corresponding offspring from the mothers not receiving the viosterol. It is evident, therefore, that viosterol exerts its influence in two ways. In the first place, vitamin D exhibits a prenatal effect on the fetus, by forcing or allowing the fetus to take more calcium and phosphorus from the maternal organism, than it would ordinarily get from the mother on a normal or limited diet. This extra drain by the fetus (under the influence of viosterol) would be expected to deplete the maternal bones of calcium and phosphorus, unless there was some protection offered to them. Therefore, in the second place, the viosterol offers this protection by causing better assimilation of the large or small amount of calcium and phosphorus in the mothers' diets, so that there is actually an over-abundance of these elements laid down in the maternal bones to counteract the excessive drain by the fetus. An interesting picture is presented in the *deficient + viosterol* group wherein the calcium and phosphorus content of the maternal bones is greater than in the *basic* group, although the calcium and phosphorus content of the diet is considerably less than in the diet of the *basic* group. However, the offspring from the *deficient + viosterol* do not have as great ash, calcium and phosphorus content as do those from the *basic* group mothers. These facts seem to indicate that the viosterol exhibits a relatively greater effect in promoting assimilation of calcium and phosphorus from the diet by the maternal organism, than in increasing the transmission of these elements from the mother to the fetus.

The only real evidence of any actual drain by the fetus on the maternal bone can be shown by comparing the *basic* and the *deficient* groups. In these groups the mothers received no viosterol. The mothers fed on the *basic* diet showed a higher content of bone calcium and phosphorus and their young showed more ash, calcium and phosphorus than did the mothers and young from the *deficient* diet. Comparing these two groups with two similar groups of *non-pregnant* female rats of the same age, and fed on the same diets for 22 days, one can see the evidence of a drain by the fetus on the ash content of the maternal bone. On analysis, the calcium content of the bone of 3 normal non pregnant females fed on the *basic* diet was 35.0 per cent, and the phosphorus content was 17.0 per cent. These values compare well with those obtained from *pregnant rats* on the *basic* diet. The calcium and phosphorus content of

of 2 normal non-pregnant rats kept on the deficient diet for 22 days was 34.2 and 16.9 per cent respectively. Comparing these values with those of the *pregnant* rats on the deficient diet, there is noted a considerable decrease in the calcium content of the bone of the pregnant rat, and this decrease is more noticeable in the phosphorus content. In the two groups of viosterol-fed rats this drain was not noticeable, since the assimilating effect of the viosterol overshadowed the lesser prenatal effect of transmission of calcium and phosphorus from the mother to the fetus.

#### SUMMARY

1 Pregnant rats fed on high and low calcium and phosphorus containing diets with added large doses of viosterol 250 D produced larger young, which contained greater amounts of ash, calcium and phosphorus than did young coming from mothers on the same diets without the viosterol.

2 The results show definite evidence of a drain on the calcium and phosphorus content of the maternal bone by the fetus only in the case of the mother rats kept on the *deficient* diet. These bones, which contained 32.5 and 14.6 per cent of calcium and phosphorus respectively, when compared to the calcium content of 34.2 and phosphorus content of 16.9 per cent as found in bones of non-pregnant females, show definite evidence of bone depletion by the fetus.

3 In pregnancy, viosterol plays a dual rôle. In the first place, it exhibits its well-known normal effect of causing better assimilation of the calcium and phosphorus in the diet by the maternal organism, and secondly, it seems to have a prenatal effect of allowing better transmission of calcium and phosphorus from the mother to the fetus.

4 Evidence of the possible interfering action of viosterol on the progress of a normal pregnancy is indicated.

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# STUDIES ON CHEYNE-STOKES RESPIRATION

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## PART I

### CHANGES IN CONCENTRATION OF GASES IN THE BLOOD

Cheyne Stokes respiration is characterized by rhythmic changes in breathing in which periods of respiratory pause alternate with periods of slowly increasing and decreasing respiratory activity. It may be accompanied by phasic variations in the functions of the heart, nervous system and other organs, and has more recently been found to be associated with periodic variation in the concentration of the gases in the blood. It occurs as a symptom in diseases of the heart and kidneys especially, in meningitis and in encephalitis, and in certain intoxications such as morphine poisoning and oxygen lack. The development of this type of breathing has been ascribed to many causes—disturbances in the gaseous exchange in the lungs, of the circulation, of the activity of the respiratory and vasomotor centers and to so called sub cortical influences. Objections to all these hypotheses have been entered with the result that no satisfactory understanding of the subject has been attained. It is of value, therefore, to record further observations of Cheyne-Stokes respiration and its attendant or resultant phenomena.

The phenomenon concerning which it seems now important to accumulate more experience, is the behavior of the gases, carbon dioxide and oxygen, in the blood under the circumstances which attend this type of respiration. For this purpose, samples of arterial blood taken from patients exhibiting this abnormality were studied. A few analyses of arterial blood have already been reported by Gollwitzer-Meier (1), Uhlenbruch (2), and Resnik and Lathrop (3). Their studies show that variations in the concentration of the gases in the blood do occur during different phases of respiration<sup>1</sup> and that at some point during the respiratory cycle, oxygen saturation of the blood usually reaches normal limits. The average carbon dioxide content in the blood is reported in some cases to be increased, in others decreased, and in others normal. In former studies only one or two samples of blood were, however, taken, but in order to ascertain the extent of the variation in the concentration of the gases in the blood during a cycle of Cheyne Stokes respiration it is

<sup>1</sup> In this paper the Cheyne Stokes cycle is described as divisible into a respiratory phase and an apneic phase.

necessary to collect from a continuous flow of arterial blood, separate, immediately successive samples as rapidly as possible. For this purpose a needle of the type usually used in puncturing arteries was connected with a nine-way stopcock\*. With its aid, samples could be rapidly obtained. A somewhat similar method has recently been published by Klem (4). The blood was collected under liquid paraffin, the oxygen and carbon dioxide content were estimated by the method described by Van Slyke and Neill (5), and the hydrogen ion concentration according to that of Hastings and Sendroy (6).

Two patients were selected for study in whom Cheyne-Stokes respiration was observed in the hospital over a period of several months, and who, because of decreased mental activity, were not disturbed by the procedure of arterial puncture. A long period of observation was regarded as important in order to be certain of the continuous presence of this type of breathing. As is well known, there are cases in which Cheyne-Stokes breathing occurs in a transient manner, as for example in cases of heart failure and morphine poisoning or in normal individuals during a sojourn in rarefied air. Since the transient type may be distinct from the continuous it is unknown at present whether the results of the observations now reported are applicable to cases of Cheyne-Stokes respiration in general.

*Case I.* The first patient, S. H., male, aged 52, Hospital number 7599, was admitted to the hospital on December 5, 1930. The diagnosis was hypertension and cardiac insufficiency. After a long period, during which he was kept under the influence of digitalis and was obliged to rest in bed, he gradually improved. For about three months before discharge the periods of Cheyne-Stokes respiration had been constantly present, but for the last three weeks, after considerable betterment, they had become intermittent. He then left this hospital.

In the only study made of this patient four samples of blood were taken, the oxygen contents of which varied between 15.1 and 18.5 volumes per cent (79.9 and 98.2 per cent of saturation), while those of the carbon dioxide varied between 42.2 and 50.1 volumes per cent. The number of samples was too small and the time during which they were taken was, in the case of each sample, too long to construct a curve, but the results are shown schematically in Figure 1. They give only an approxi-

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\* This stopcock was made of glass and first used in January 1931. Blood entered through an opening in the bottom of the center plug emerging on the side. Light perforations through the jacket in a circle at the level of the opening in the center plug lead by rubber connections to openings into the lower ends of the glass sample tubes. The sample tubes are held by a stout rubber band against the outer surface of the jacket. The whole apparatus is then filled with paraffin oil. By revolving the jacket with the sample tubes fixed to it the tubes can be successively and rapidly connected to the arterial needle or cannula.

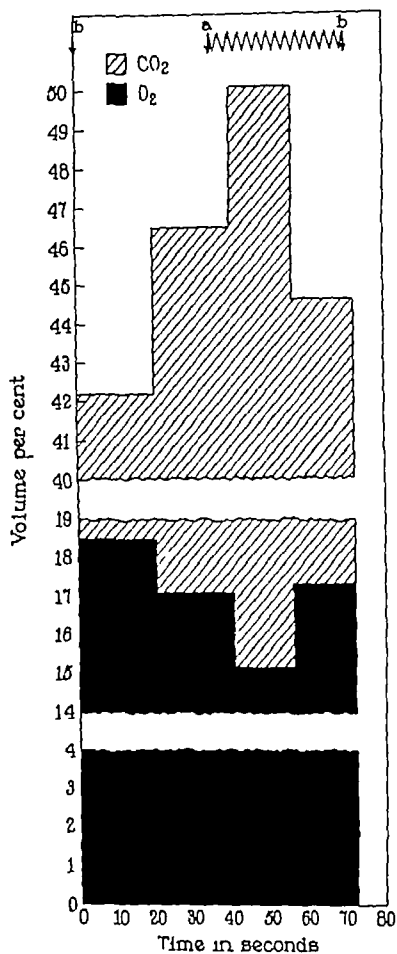


FIG 1 CHART OF THE VARIATIONS IN CONCENTRATION OF OXYGEN AND CARBON DIOXIDE IN BLOOD OBTAINED FROM THE RADIAL ARTERY DURING A CYCLE OF CHEYNE STOKES RESPIRATION (CASE I)

Arrows "a" mark the onset of dyspnea, arrows "b" the onset of apnea



mate account of what the course of events must have been. The true maxima and minima were undoubtedly greater than the levels actually obtained in the samples, but whatever their magnitude they were probably attained within the period of time during which the samples characterized by the extreme values were collected. The greatest oxygen concentration was found to occur during the first half of apnea. It decreased during the second half of apnea and the first part of the respiratory phase wherein it reached its minimum and began to increase again. The maximum was reached as before at the beginning of apnea. The concentration of carbon dioxide varied in an inverse direction. The mean concentrations of both gases occurred at about the beginning of the respiratory phase and either mean concentrations or values between this level and the maximal oxygen and minimal carbon dioxide concentrations occurred at the end of the respiratory phase.

*Case II.* In the second patient, S. G., male, aged 56, Hospital number 7745, admitted April 24, 1931, there had developed first right, then left hemiplegia several years before admission. Pareses of all the extremities, and aphasia still persisted. Arterial hypertension was present, but there were no signs of cardiac insufficiency. In the first study (Figure 2) four samples of blood during a single cycle were secured, and one sample during the whole of the next succeeding one. By superimposing the results obtained during the first cycle upon those of the second it becomes obvious that the longer the time consumed in collecting a single sample, the greater the deviation of the values found from what must have been the actual or extreme values. The oxygen content varied only between 16.1 and 16.9 volumes per cent (89.1 and 93.0 per cent of saturation) and the carbon dioxide between 40.8 and 45.6 volumes per cent. That in this instance the range of variation in the concentration of the gases in the blood was so much less than in Case I may have been due to the fact that the phases of apnea and dyspnea were shorter. The oxygen minimum lay, however, at the same point as in Case I, but the maximum was found to fall, not in the first part of the apneic phase but during the last few respirations. Respiration therefore stopped when maximal oxygen and minimal carbon dioxide concentrations were reached, while it began when they attained mean values. The oxygen content furthermore, decreased more slowly than it increased. The hydrogen ion concentration of the blood varied with the changes in concentration of the gases, reaching the peak of acidity (pH 7.40) at the time of the carbon dioxide maximum, and decreasing to pH 7.44 at that of the carbon dioxide minimum.

In the second study of this patient (Figure 3), eight samples of blood were taken during two cycles of Cheyne-Stokes breathing. The oxygen content varied between 16.6 and 18.2 volumes per cent (83.7 per cent and 98.7 per cent of saturation), the carbon dioxide between 40.5 and

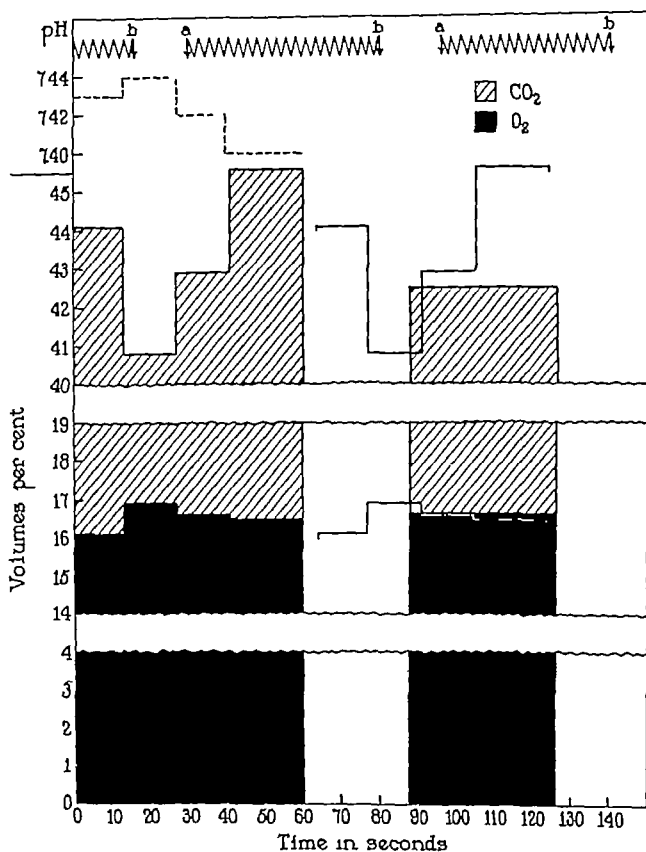


FIG 2 CHART OF THE VARIATIONS IN CONCENTRATION OF OXYGEN AND CARBON DIOXIDE IN BLOOD OBTAINED FROM THE RADIAL ARTERY DURING A CYCLE OF CHEYNE STOKES RESPIRATION (CASE II)

A fifth sample of long duration obtained during the next succeeding cycle illustrates the "leveling" effect. The solid line represents the changes in gases of the blood found during the first cycle reproduced in chronological relation to the second cycle. The broken line traces the change in hydrogen ion concentration.

45.1 volumes per cent. That these changes were greater than those obtained on the previous occasion may have been due to the greater length of the Cheyne-Stokes cycles, or to the shorter time during which each sample was collected. The time at which carbon dioxide was at its maximum and oxygen at its minimum bore the same relation to the

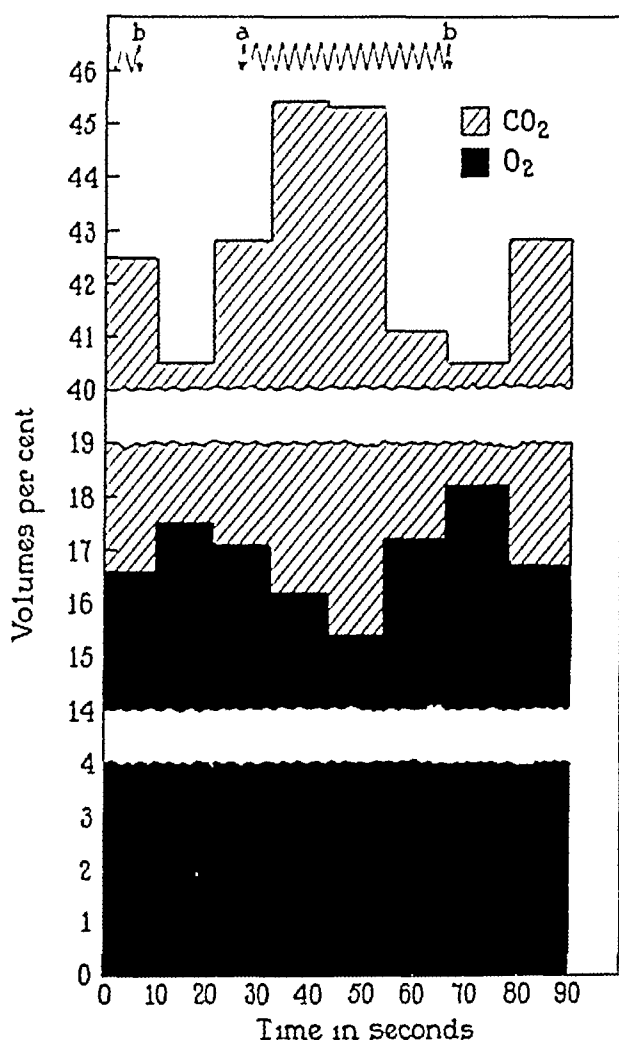


FIG. 3. CHART OF THE VARIATION OF THE GASES IN BLOOD OBTAINED IN A LATER STUDY ON THE PATIENT SHOWN IN FIGURE 2

respiratory cycle as in the previous observation in this patient. The oxygen maximum lay in the first part of apnea. Concentrations of both gases were approximately near the mid-level when the respiratory phase began. In this study too, oxygen concentration decreased and carbon dioxide concentration increased more slowly than these increased and decreased respectively.

## DISCUSSION

In these observations the minimal concentration of oxygen in blood taken from the radial artery occurs constantly during the respiratory phase of the Cheyne Stokes cycle, and the maximum at the end of this phase, or at the beginning of apnea. The maximal and minimal concentrations of the carbon dioxide occur at the time of the minimal and maximal concentrations respectively of oxygen. Similar results have been reported by Klein (4).

In order to set forth accurately the relations in time between the Cheyne Stokes cycle and the concentration of gases in the blood leaving the lungs, one must recall the fact that any given concentration at this site precedes its arrival at the radial artery by the time of transmission, which being but two or three seconds, changes the relations only by insignificant amounts. By referring to Figure 3, it becomes apparent that the first few small respirations have little effect on the gases of the blood. The peak of saturation with oxygen is attained, moreover, just before or immediately upon the end of respiration, and indicates that the last few respirations also are quite ineffective.

Because of the different conditions attending the exchange of oxygen and of carbon dioxide between blood and lungs, the changes in their concentrations, though always opposite in direction, are not always inversely proportional. Aside from this relation, the amount of each gas present influences, as is well known, the concentration of the other. The question arises, therefore, how far the concentrations of gases in the blood are determined by variations in alveolar tension, and how far by the varying concentrations of the gases present in the blood itself. This problem has its importance, but the facts which are known are insufficient to analyze the processes which are involved.

The concentration of hydrogen ions in the blood shows changes consistent with the variations found in that of carbon dioxide. These variations are, however, relatively speaking small, due to the presence of the usual buffers of the blood. Since it is known that hemoglobin takes up one half molecule of carbon dioxide for each molecule of oxygen lost, without change in hydrogen ion concentration, the actual range of variation is still smaller than might be expected because decrease in oxygen takes place simultaneously with increase in carbon dioxide.

If changes in concentration of gases or of hydrogen ions in the blood control phases in the Cheyne-Stokes cycle, the levels of their various concentrations at the beginning of the respiratory phase take on new significance because it is at this point that, considered collectively as a stimulus, they may be assumed to have reached the threshold value of the respiratory center. Should this suggestion concerning the chain of events be correct, it would afford in man an opportunity to ascertain the threshold value of the respiratory center. The importance of know-

ing this value lies in the fact that it indicates a definite stage of irritability, readily discerned by a change from inactivity to activity in the organs controlled by the center. If the composition of the blood which is taken from the radial artery is identical with that which arrives at a corresponding time at the respiratory center (due regard being paid, of course, to differences in distance and in the nature of the arterial channels), the threshold of irritability of the respiratory center to carbon dioxide and oxygen lies at about the mean concentration of these gases in the blood, and also at the mean value of the hydrogen ion concentration, between pH 7.40 and 7.44. The last respiration may be a sign, furthermore, that the stimulus derived from the concentration of the gases in the blood has fallen below the threshold value. The impression has been gained in the course of these observations that the threshold changes, that it is lower when the intensity of the stimulus derived from the blood is decreasing than when it is increasing. The problem of the function of the respiratory center with regard to stimuli of increasing and decreasing strength has been so little studied that for the moment further discussion seems unprofitable.

From studies of the analyses of the gases in the blood alone, an explanation of the mechanism of Cheyne-Stokes respiration is not to be expected. For this reason objections may be raised to the conclusions of Klein. His observations and the results he obtained from them are, however, in close accord with those now described. By locating, in the phases of the Cheyne-Stokes cycle the instants of maximal and minimal saturation of oxygen in blood taken from the radial artery, he demonstrated that the oxygen saturation was lowest at about the height of dyspnea and greatest at the beginning of apnea. He then showed, by means of injections of Congo-red into an arm vein that, in patients with Cheyne-Stokes breathing, 18 to 22 seconds passed before the dye appeared in the radial artery, while in normal individuals only 8 to 12 seconds were required. From the increased time of flow he calculated the time at which the blood in the lungs would be most and when least saturated with oxygen. Maximal saturation (in the lungs) occurred at or just before the height of the respiratory phase, but breathing continued until blood saturated with oxygen arrived at the respiratory center. Respiration then stopped. The response of the respiratory center delayed by the slow rate of circulation shown by the experiments with the dye with respect to conditions at the site of ventilation (the lungs) he terms "Nachhinken." After complete saturation of the blood in the respiratory phase, over-ventilation, he believes, occurs with loss of carbon dioxide. Apnea then follows. If the time required for blood to flow from lungs to center is so important a factor as believed by Klein in maintaining periodicity of respiration, changes in the rate of flow should alter the length of the respiratory cycles. But this relation does not exist. If it

did, the breathing of normal persons should be periodic in character, the cycles, of course, being shorter since the time required for their blood to reach the respiratory center is but half that found by Klein in Cheyne-Stokes respiration. There are, besides, cases in which the time of flow is prolonged without bringing on Cheyne-Stokes respiration. If a decreased rate of flow were, moreover, responsible for cyclic breathing, the method by which the cycles should disappear is by becoming shorter and shorter. This, however, is not the case in our studies. When increased concentrations of carbon dioxide were administered during Cheyne Stokes respiration, the cycles remained of about the same length and their disappearance was occasioned by gradual decrease in the intensity of the mid point of the respiratory phase and by the gradual encroachment of this phase upon the phase of apnea. When, on the other hand, oxygen was administered, the cycles attained twice the usual duration. There is no evidence at hand, however, to show that the circulation time is prolonged by oxygen. Uhlenbruck also speaks of "Nachhinken" of the respiratory center as a result of local difficulties in circulation or in the diffusion of gases.

If, instead of assuming that the time of arrival of blood at the respiratory center and at the radial artery are simultaneous, various intervals of time are assumed and the resulting relations between the beginning of respiration and the concentrations of gases in the blood at the respiratory center are examined, it becomes possible to distinguish on a curve representing changing values of the stimulus of the blood, which portion is capable of arousing the respiratory center. In these observations at the beginning of the respiratory phase the blood in the radial artery was found to have a composition represented on this curve by "a" (Figure 4). Should blood leaving the lungs at the same time, and having the same composition, not arrive at the respiratory center until, for example, about 15 seconds later, it would have the same composition as that obtained from the radial artery 15 seconds before the respiratory phase began, that is to say, one similar to "b", or, should the difference in time be greater, a composition similar to "c" or "d". But these assumptions are all physiologically improbable since blood of low carbon dioxide concentration having weak stimulating value, would then be associated with respiratory activity, while that of much greater concentration of carbon dioxide, with apnea. On this calculation the arrival of blood of identical composition at the radial artery and at the respiratory center cannot differ by more than 10 or 15 seconds.

Should blood from the capillaries of the lungs take, for example, about 15 seconds to reach the radial artery it would have at the beginning of respiration the same composition as that obtained from the radial artery 15 seconds after respiration began, that is to say a composition similar to "b'" (Figure 4). But this relation is likewise physiologically

improbable because the blood in the lungs would then exhibit the effects of ventilation (decrease in content of carbon dioxide) before respiration began. Such a situation is illustrated in Figure 4 where "b'" would necessarily represent the blood in the lungs at the beginning of the respiratory phase if it took as long as 15 seconds to reach the radial artery. The interval of time cannot be greater than this in our observations. From the location of these limits it becomes apparent that the values actually found to be associated with the onset of the respiratory phase lie in the mid-zone of the portion of the curve which is capable of arousing the respiratory center.

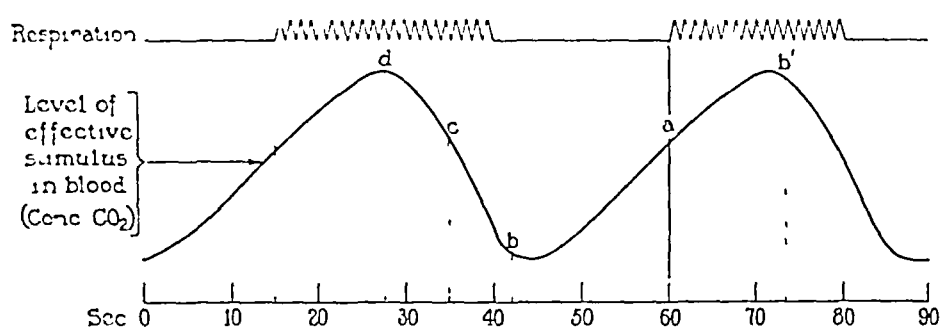


FIG. 4. SCHEMATIC REPRESENTATION OF VARIOUS RELATIONS OF THE LEVEL OF STIMULUS IN THE BLOOD (CONCENTRATION OF CARBON DIOXIDE), TO THE PHASES OF THE CHEYNE-STOKES CYCLE.

The time relations as drawn are based on the composition of blood obtained from the radial artery. Imagine the respiratory curve shifted to the right so that the beginning of the respiratory phase falls at *b'*, 13 seconds after *a* instead of at *a* in order to obtain the relation between blood in the capillaries of the lungs to the phases of respiration if the time of blood flow from lungs to radial artery were as great as 13 seconds or to the left to *b*, *c* or *d*, in order to obtain the relation between blood in the respiratory center and the phases of respiration if blood took 18, 25 or 32 seconds longer to reach the medulla than the radial artery.

#### SUMMARY

By collecting a series of samples in immediate succession and during the briefest periods consistent with securing amounts sufficient for analysis from the continuous stream of blood flowing from the radial artery it has been possible to analyze the influence of Cheyne-Stokes breathing on the concentration of the gases in the blood.

In the blood drawn from the radial artery, maximal oxygen and minimal carbon dioxide concentrations occur during the end of the dyspneic or first part of the apneic phase. The minimal oxygen and maximal carbon dioxide concentrations occur during the earlier part of the respiratory phase. At the beginning of active respiration, the gas concentrations lie at about a mean value.

## PART II

## OBSERVATIONS ON THE EFFECT OF INHALING OXYGEN AND CARBON DIOXIDE

Oxygen lack of the respiratory center is often regarded as the cause of Cheyne Stokes breathing. This condition is ascribed in some cases to a lack of oxygenation of the blood in the lungs, in others to anoxemia produced by undue slowing of the general or local circulation, and in still others to difficulty in the passage of blood in the respiratory center. Opinions such as these are based on observations of Cheyne Stokes breathing in normal persons at great elevations above sea level, on Cheyne Stokes respiration brought about by the inhalation of gas mixtures low in oxygen and on clinical investigations of persons exhibiting the Cheyne Stokes syndrome. The theory is supported, furthermore, by reports of improvement in condition following the administration of oxygen. Several authors (7, 8) have described cases in which Cheyne-Stokes respiration was brought to an end and regular breathing reestablished by the inhalation of oxygen as well as by the inhalation of carbon dioxide. Recently, Uhlenbruck (2) reported that the result with oxygen was transient, regular breathing occurring during the first few moments of administration only. Because the theory of oxygen lack is important both in attempting to analyze the nature, and in the therapy of Cheyne Stokes respiration, its effect on the form of Cheyne-Stokes breathing was studied in detail.

The administration of oxygen was effected in some instances by inhaling from a mask connected through a rubber bag with an oxygen tank, in others, by enclosing the patient in an oxygen tent. The most satisfactory method was to place the patient in an oxygen chamber. We place special emphasis on the long duration of observation which the last mentioned method permits. Observations made when a mask is used are unsatisfactory because of increase in the dead space and the consequent possibility of rebreathing carbon dioxide, and also because of the danger of leak about the mask. Another objection results because patients sometimes change the type of breathing when a mask is applied to the face. Records of respiration were made by a Marey tambour connected by tubing with two rubber bags held in place against the chest by a canvas belt. The transmission was by air. The lever of the tambour wrote in ink on glazed paper moved by a revolving drum. The tracings were used only in calculating time relations, for volumetric measurements they were useless. To secure volume curves a Roth Benedict spirometer and mask were used. The objections to this method are the same as those mentioned above. A more serious objection, due to progressive decrease in oxygen content of the gas in the spirometer, was overcome by increasing the total volume of the apparatus to about 20 liters. This large capacity was obtained by joining several large glass



bottles in series in the respiratory circuit. Oxygen variations under these conditions were less than 3 per cent.

Before the administration of oxygen, records were made of the patients in room air over a period of several hours. In some patients Cheyne-Stokes breathing persisted continuously during this period, in others, it alternated with periods of regular respiration. Transitions between the two were frequently observed in passing between the waking and the sleeping state (Figures 5*a* and *b*). In studying the effect of inhalation

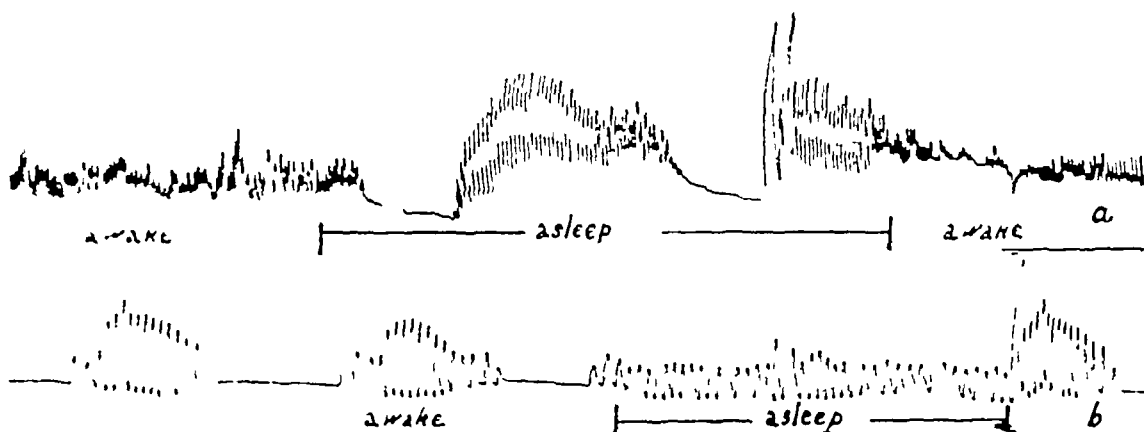


FIG. 5. KYMOGRAPHIC TRACINGS OF TRANSITION TO (*a*) CHEYNE-STOKES RESPIRATION ON FALLING ASLEEP AND (*b*) IN ANOTHER INDIVIDUAL TO CONTINUOUS BREATHING ON FALLING ASLEEP.

In this figure as well as in Figures 6, 7, 8, 9 and 12 the tracings have been reduced to four-fifths of their original size.

of oxygen only patients who were subject to continuous Cheyne-Stokes breathing were observed, except one in whom this type of breathing was present constantly while he was awake.

Carbon dioxide was first administered to some patients in order to secure curves to be used in comparison with those obtained when oxygen was used. Inhalation of 5 per cent carbon dioxide by mask stopped the occurrence of apneic phases after two minutes, only the frequency of respiration then showed periodic variations. As soon as carbon dioxide was withdrawn, Cheyne-Stokes respiration recurred. No after effects suggesting exhaustion of the respiratory center appeared. In an experiment of long duration, carbon dioxide was administered in a gas-tight chamber in which the gas could be slowly increased and accurately analyzed and in which the oxygen content was maintained practically constant. By this means the details of the onset of continuous breathing were recorded. A period of control is illustrated in Figure 6*a*. When the carbon dioxide content reached one per cent, the respiratory phases began to increase in duration (Figure 6*b*) while the apneic phases became

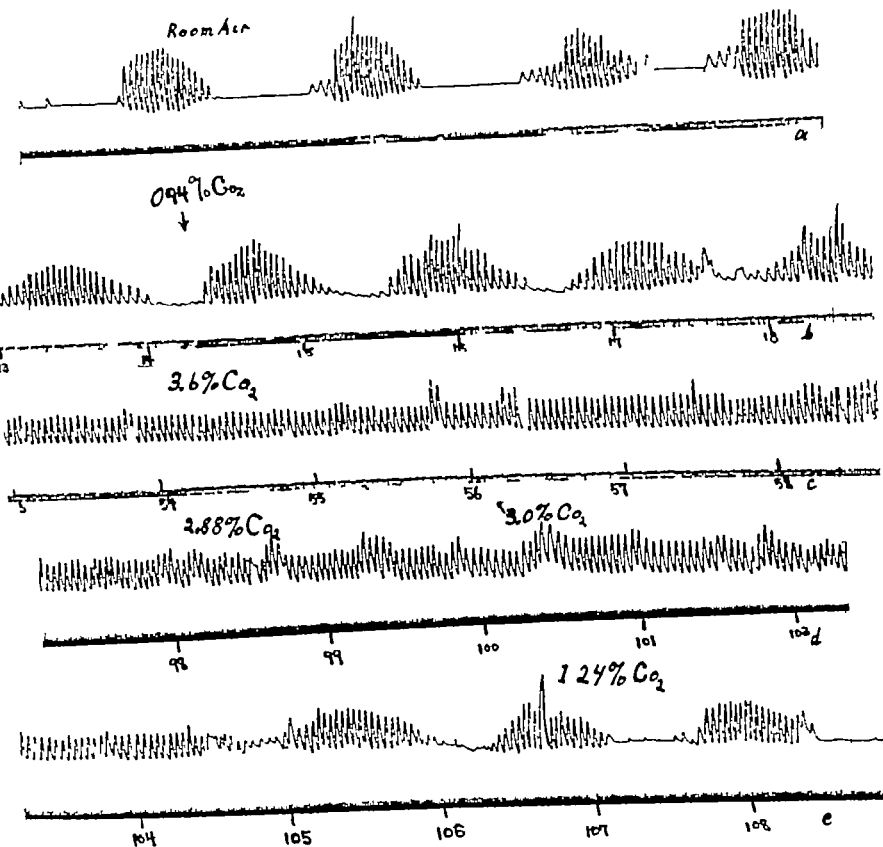


FIG 6 TRACINGS OF CHANGES IN FORM OF RESPIRATION SHOWING THE GRADUAL DEVELOPMENT OF CONTINUOUS BREATHING DURING THE INHALATION OF INCREASED AMOUNTS OF CARBON DIOXIDE

Note the gradual encroachment of the dyspneic upon the apneic phase in *b* causing a disappearance of the latter without change in the duration of the whole cycle

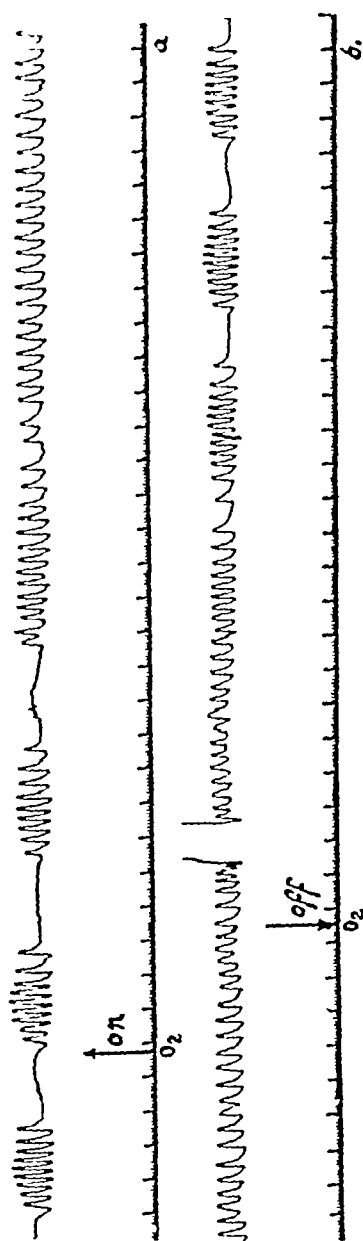


FIG. 7. THIS TRACING SHOWS THE ONLY EXAMPLE OF CONTINUOUS BREATHING OBSERVED DURING INHALATION OF OXYGEN WHICH, IN THIS INSTANCE, WAS ADMINISTERED BY A MASK.

shorter and began indeed to disappear. The length of an entire cycle did not, however, increase. When the concentration reached approximately 3 per cent (Figure 6c and d), the breathing was continuous and remained so throughout the whole period of administration lasting one and one-half hours. On discontinuing the administration of carbon dioxide, the transition to Cheyne-Stokes breathing took place. The apneic phases were at first brief, then grew longer, while the respiratory phases grew shorter (Figure 6e). No after effect suggesting exhaustion of the respiratory center was apparent even after this prolonged period of inhalation.

Once when oxygen was administered for a short time by mask there was a sudden transition to continuous breathing after two minutes (Figure 7). On another day, using the same technique, continuous breathing did not appear but the respiratory phase increased from 37 to 67 seconds, while the duration of apnea did not change appreciably. The whole Cheyne-Stokes cycle, therefore, increased about 50 per cent. A somewhat similar increase in another instance occurred when a mask fitted with one-way valves was used, and 80 per cent oxygen was inhaled from a large spirometer (Figure 8a). In this case a marked increase in

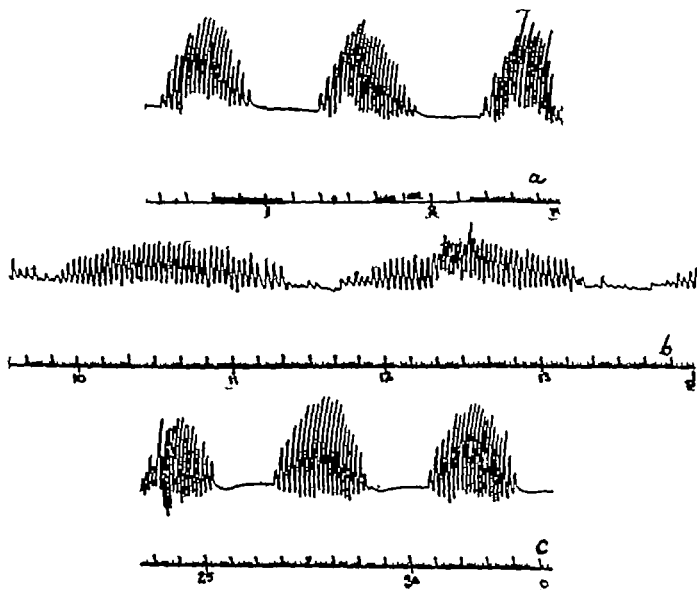


FIG 8 TRACINGS *a* AND *c* WERE OBTAINED BEFORE AND AFTER, AND *b* DURING THE ADMINISTRATION OF 80 PER CENT OXYGEN BY MASK AND VALVE.

The increase in the length of the whole cycle is well shown in "*b*"

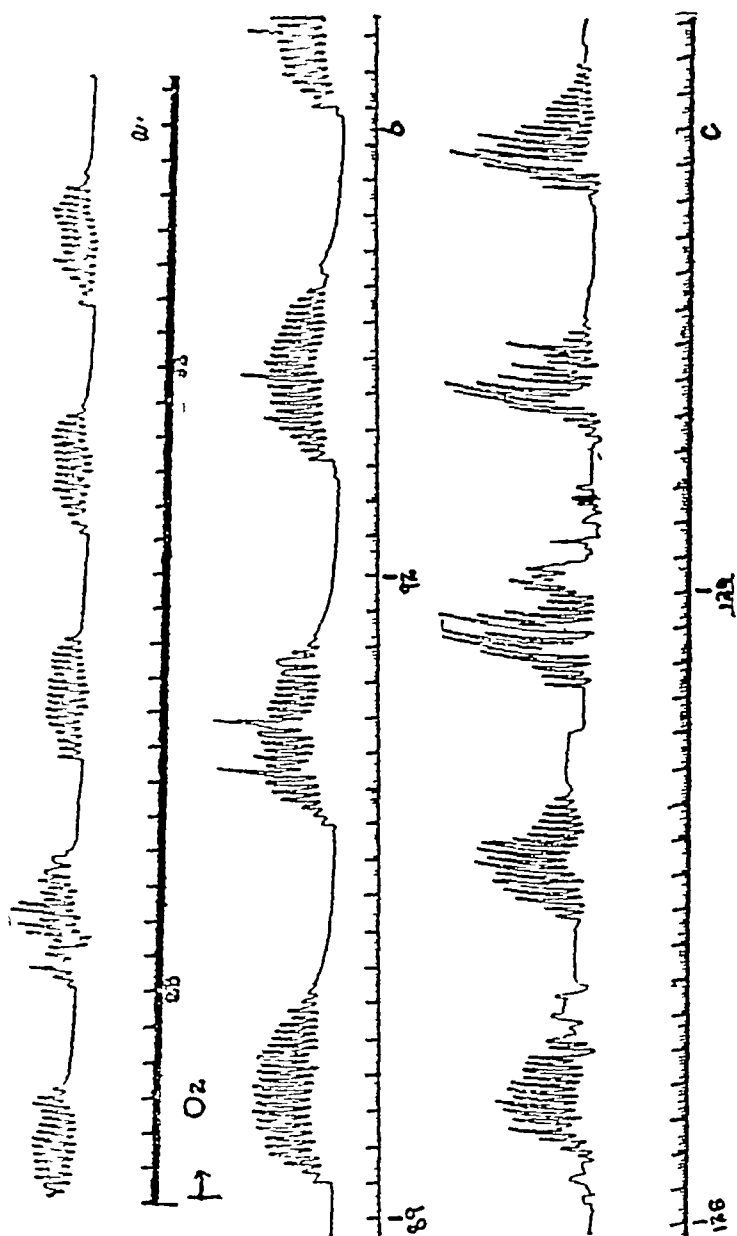


FIG 9 TRACINGS *a*, *b* AND *c* WERE TAKEN BEFORE, DURING, AND AFTER THE ADMINISTRATION OF OXYGEN UNDER A TENT

The whole cycle has increased as in Figure 8*b*, but the increase in this instance is mainly due to the greater duration of apnea

the length of the respiratory phase and a decrease in the duration of apnea took place, the whole cycle increasing 75 per cent in length (Figure 86) In the oxygen tent and chamber where the content of oxygen in the air breathed ranged from 40 to 55 per cent, somewhat similar results were obtained except that a decrease in the duration of apnea was never observed When duration of apnea changed, it always increased in length An increase in the length of the respiratory phase was always present (Figures 9, 10 and 11)

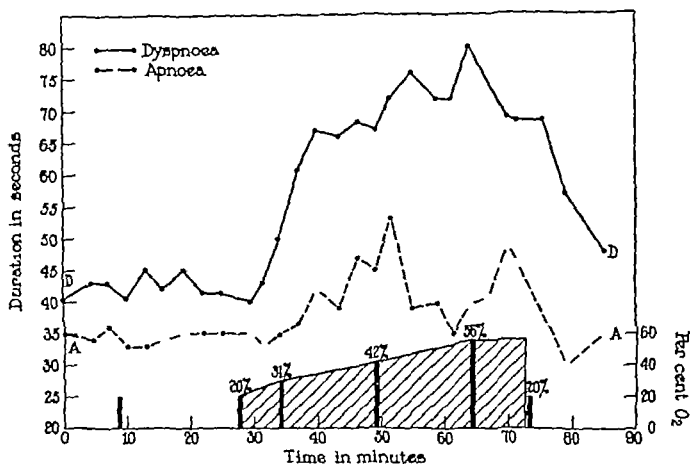


FIG 10 CHART OF THE CHANGE IN DURATION OF APNEA AND DYSPNEA DURING INCREASE IN THE CONCENTRATION OF OXYGEN INHALED

In one patient curves of the respiratory volume were obtained while the concentration of oxygen of the respired air was changed from 20 to 33 per cent (Figure 12) The respiratory and apneic phases both increased as usual, while the respiratory volume per minute decreased about 10 per cent, an amount just within the limit of error of measurement. The total amount of air respired during one whole cycle increased, however, about 50 per cent. This result was due to increase in length of the respiratory phase as well as to increase in the volume of each individual respiration By modifying the cycles of his breathing a patient suffering from Cheyne-Stokes respiration may alter, without changing the volume of respired air, the composition of the gases in the blood It is improbable therefore that measurement of the volume of the respired air would afford reliable information about the oxygen absorbed or the concentration of the gases in the blood

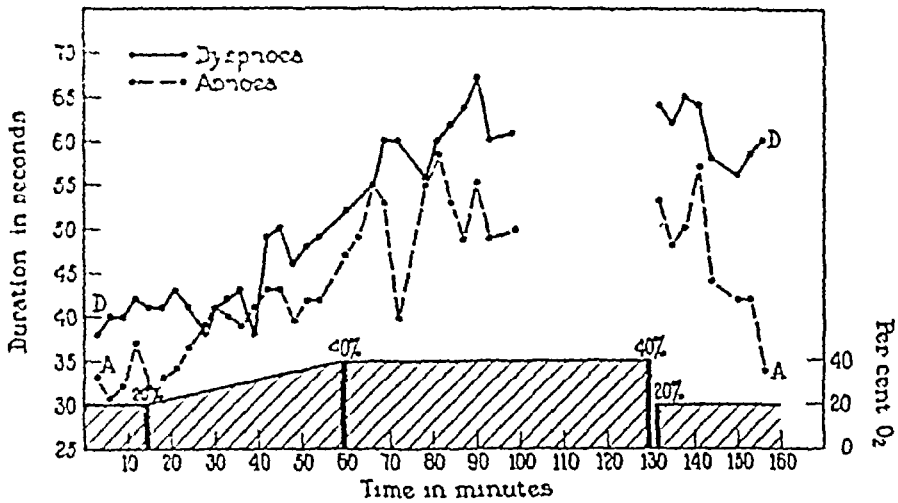


FIG 11 CHART OF THE CHANGE IN DURATION OF APNEA AND DYSPNEA DURING INCREASE IN THE CONCENTRATION OF OXYGEN INHALED

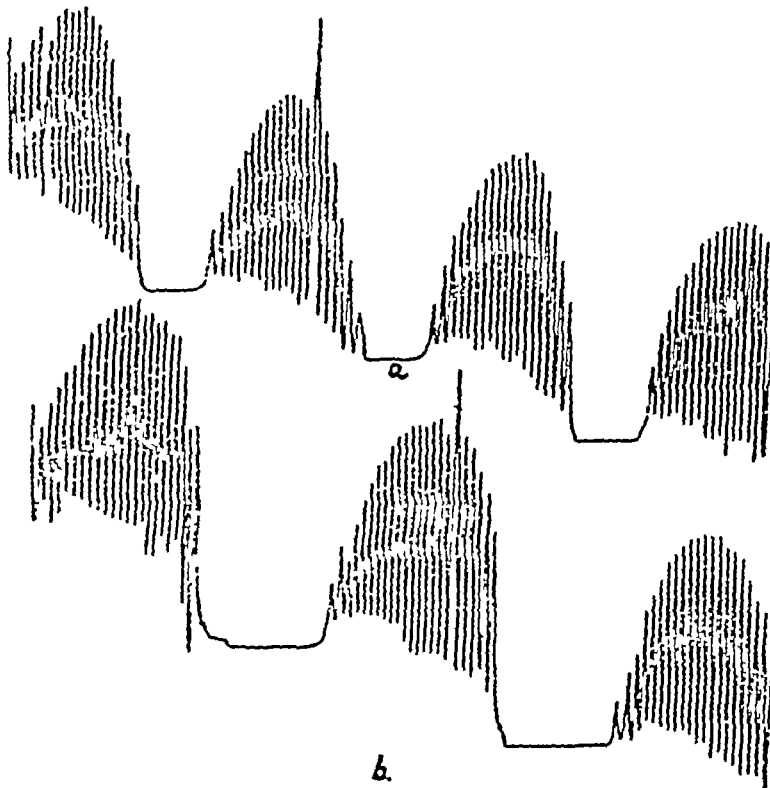


FIG 12 VOLUMETRIC TRACING OF RESPIRATION (BENEDICT-ROTH MODIFIED) (a) BEFORE AND (b) DURING INHALATION OF INCREASED CONCENTRATION OF OXYGEN

A series of samples of arterial blood were examined during the administration of oxygen for comparison with the results obtained when room air was respired. During its administration the variations in concentration of both oxygen and carbon dioxide in the blood were much less during a respiratory cycle. In none of the samples did the content of oxygen fall below 90 per cent even though the cyclic form of breathing did not cease.

### DISCUSSION

In the present study increase of carbon dioxide content of the atmosphere to which patients, the subjects of Cheyne-Stokes breathing, were exposed, consistently brought about continuous respiration. A similar result was not obtained by the administration of oxygen even if the concentration was increased up to 80 per cent.

With carbon dioxide, the respiratory phase increased and the apneic phase decreased in length, so that the duration of the whole cycle remained unchanged. With oxygen, on the other hand, the length of the respiratory phase increased while that of apnea remained the same or became even longer, so that the duration of the whole cycle was greatly prolonged. That continuous breathing may follow the administration of high percentage of oxygen when given by means of a mask may be due to factors suggested earlier in the paper as objections to this method of administration. It may be due, however, to conditions inherent in the experiment which differ from those obtaining in the use of the oxygen tent or chamber, namely, the occurrence of much greater concentrations of oxygen or the abruptness of the change from atmospheric to higher concentrations of oxygen. Further investigations of the conditions of administration are necessary. These observations do not, we think, permit the inference which has been drawn by others, that inhalation of increased concentrations of oxygen stops Cheyne-Stokes breathing. It is possible, however, to confirm that part of the results of Uhlenbruch's experiences which show that the form of Cheyne-Stokes respiration can be modified by a change in concentration of the gases which are inhaled. It is impossible, therefore, to regard the periodicity of Cheyne-Stokes breathing as an expression of the peculiar rhythm of the respiratory center alone since this periodicity may be modified or even arrested by changing the composition of the inhaled air.

That inhalation of percentages of carbon dioxide higher than normal brings about continuous breathing is probably due to the fact that no matter how great the degree of dyspnea, the concentration of carbon dioxide in the alveolar air prevents the escape of carbon dioxide from the blood in sufficient quantities to allow the stimulating properties of the blood to fall below the threshold value for the respiratory center. Administration of a concentration of carbon dioxide just sufficient to occasion



continuous respiration might, therefore, constitute another measure of the irritability of the respiratory center. An analysis of the effect of breathing high percentages of oxygen is more complicated because two factors are involved. In the first place oxygen lack is generally regarded as a respiratory stimulus. When more of this gas is made available, the strength of the stimulus, therefore, decreases, but the stimulating property of the blood may, nevertheless, be increased as the result of oxygenation, since oxyhemoglobin develops stronger acid properties than hemoglobin (9, 10). The two factors act in opposite directions. For instance, by eliminating oxygen lack, one might suppose that the apneic phase would be prolonged, the respiratory phase shortened, and the depth of ventilation decreased. But by reason of the greater acid property of oxyhemoglobin, the effect of better oxygenation might be similar to that of breathing carbon dioxide in that it might result in an increase in the acidity of the blood. Analysis of the nature of the mechanism is also complicated by the fact that every change in the relations of the phases of the Cheyne-Stokes cycle occasions change in the carbon dioxide content of the blood. Further studies of the behavior of the gases in the blood are necessary in the attempt to solve this problem.

If the treatment of Cheyne-Stokes respiration has as its object the restoration to normal of the concentrations of the gases in the blood, then bringing about continuous respiration need not of itself be regarded as improvement, because continuous breathing alone does not necessarily mean that the ventilation is more nearly sufficient. For example, two patients with Cheyne-Stokes respiration were observed in whom the last stage of their disease was accompanied by an increase in cyanosis, and return to continuous breathing at one and the same time. If the object of treatment is to bring about continuous respiration, then inhalation of carbon dioxide in a chamber may be attempted, using the smallest concentration necessary to assure this result. To decrease the rhythmic recurrence of lack of oxygen, the effect of inhalation of oxygen in the chamber may also be utilized as is shown by the analysis of blood of patients while they are in the oxygen chamber.

#### SUMMARY

The influence on Cheyne-Stokes respiration of breathing varied mixtures of carbon dioxide and air has been studied in periods both of short and of long duration.

Increase in concentration of carbon dioxide in the air inhaled prolongs the respiratory phase, and decreases the apneic phase until continuous breathing appears.

Increase in the concentration of oxygen up to 80 per cent in the air inhaled prolongs the respiratory phase markedly. The duration of apnea is sometimes increased, and sometimes remains constant. Inhaling

oxygen in greater concentrations than that of air does not usually result in continuous breathing

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